The Journal of Parasitology

Volume 22 AUGUST, 1936 Number 4

FURTHER OBSERVATIONS ON THE BEHAVIOUR OF *WOHLFAHRTIA VIGIL* (WALK.) WITH NOTES ON THE COLLECTING AND REARING OF THE FLIES

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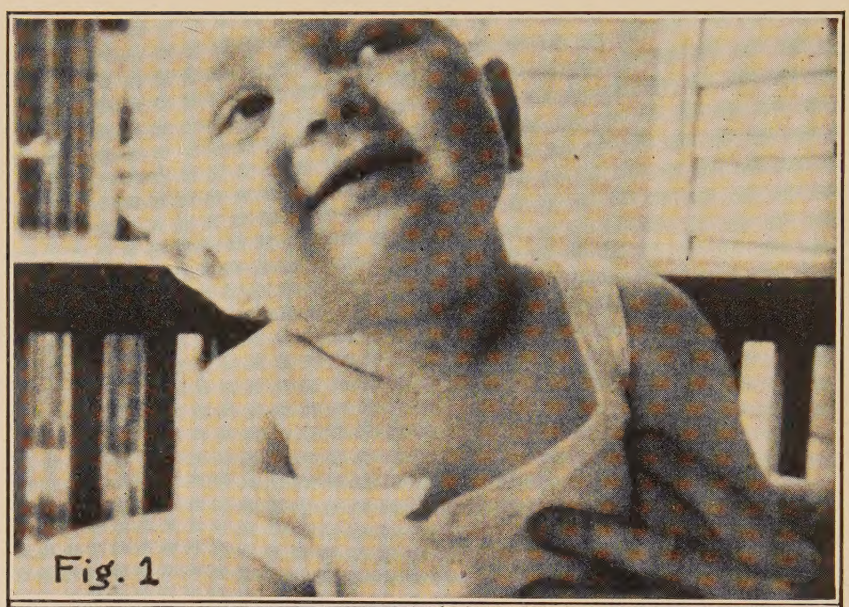
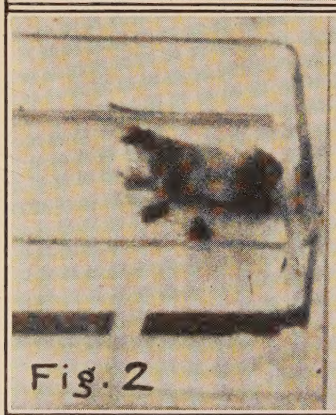
Three years ago the writer (Ford, 1932) published some observations on the behaviour of the sarcophagid fly, *Wohlfahrtia vigil,* the individuals studied having been reared from a case of cutaneous myiasis reported from the Sick Children's Hospital in Toronto in June 1931. At that time we were interested in the feeding habits of the flies, such as their avoidance of carrion and their decided preference for sugar, nectar of flowers and fruit juices; we emphasized also the behaviour reactions of the female when at the time of larviposition she was attracted to the eye of the host.

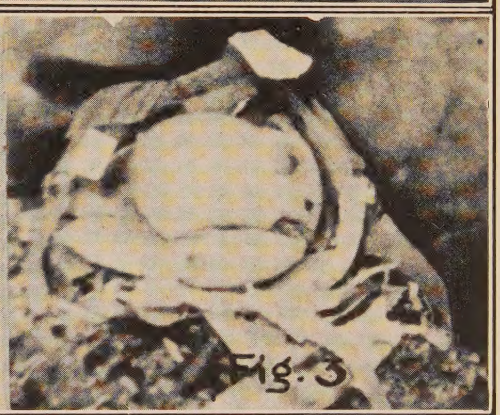
Since the summer of 1931 we have been watching for wohlfahrtias in the field and these have now been found in five localities near Toronto in the years 1933, 1934 and 1935, and at Bayfield on Lake Huron in August 1935. Four additional cases of myiasis in infants occurred in the city early in the summer of 1934 and a large number of cases among young animals. We have therefore been able to rear a great many flies, to carry out some experimental work and to take moving pictures of their behaviour towards their hosts and their feeding in the field. Puparia and larvae were put away at low temperatures to be used whenever needed. Controlled emergence of the adults was obtained at any time, summer or winter, during 1933 and 1934, but was a failure in 1935.

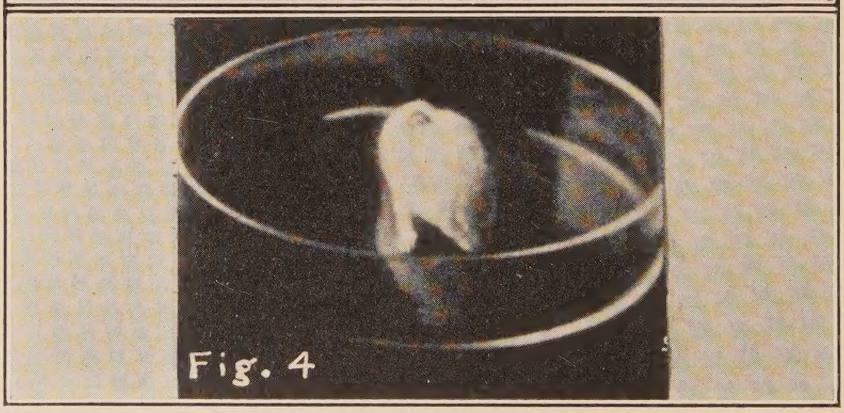
Four Cases of Myiasis in Children in 1934

On June 12, 15, 22 and 23, 1934, four cases of myiasis in babies in Toronto were reported to Dr. E. M. Walker and the writer. Three of the infants were in the Hospital for Sick Children and accounts of their cases have been published by Dr. N. Silverthorne and Dr. Alan Brown (1934). Through the kindness of Dr. Brown and Dr. Silverthorne we were able to rear the larvae in each instance and to take motion pictures of the children as a part of the general record of our work on *Wohl-*









All figures are enlargements from 16 mm. ciné-film.

fahrtia. The fourth case was reported to us by Dr. J. H. McClelland and again the larvae were reared and pictures taken.

Photographs of the first three cases have not been included in the present paper since they are illustrated by Silverthorne and Brown (1934).

Case I. K. E. Hospital for Sick Children. The child, 3 weeks old, was admitted to the hospital on June 9, having had a rash on the arm and neck for 1 day and spots appearing on the day of admission. On June 11 a larva was removed with forceps from an area on the side of the neck. "A definite small purulent sinus was left, surrounded by a red and quite indurated margin." (Dr. Silverthorne's Report). The maggot was placed on raw beef and the following day measured 13 mm. On June 15 it pupated and on July 1 the fly emerged.

Case II. P. P. Hospital for Sick Children. A baby of 3 months was admitted to hospital on June 14, having had red spots under the eye and ear for 2 days. From each area several small maggots were extracted. These were placed on rabbit muscle and on June 15 measured 5 mm. Only 2 of the larvae were uninjured and grew. On June 16 they measured 10 mm.; on June 17, 15 mm.; on June 18 pupated in sawdust; on July 4 an adult fly emerged.

Case III. D. G. Hospital for Sick Children. A child, 5 weeks old, was admitted to hospital on June 22. This was the most serious case, the hand and arm of the baby being in a horrible condition. Dr. S. G. Parker, the personal physician, stated that the 12 or 15 lesions had been noticed the day before, when they had the appearance of mosquito bites, except that each bite seemed to have a purulent centre. On the day the child was taken to hospital the lesions began to swell, pus was discharged and the larvae were clearly visible, a group of four or five being in many cases crowded in one lesion, with the posterior ends of the larvae pulsating rhythmically with their feeding movements. Between thirty and forty larvae were removed. On June 23 these measured 10 mm.; on June 24, 15 mm.; on June 27 they entered the sawdust; on July 9, 10 and 12 the flies emerged.

Case IV. G. Physician, Dr. J. H. McClelland. (Fig. 1.) A baby, 9 months old, had been sleeping on the front lawn under a small maple tree in the late afternoon of June 22nd. When the mother picked the baby up she noticed a mass of larvae on its throat and some of these

FIG. 1. Infant, nine months old; throat showing ten to twelve bright red spots 24 hours after removal of first instar larvae of *Wohlfahrtia vigil* (Walk.) which were burrowing into the skin.

FIG. 2. Three females of *Wohlfahrtia vigil* about to larviposit on a new-born rat. One fly on the head of the animal.

FIG. 3. Adult mouse removing larvae of *W. vigil* from lesion on offspring.

FIG. 4. Twenty-four hours later an additional lesion had been made by the mother on the back of the young mouse, in an attempt to remove the larvae.

were penetrating the skin. The mother brushed the larvae off and pulled out with tweezers those which were burrowing. In the evening of the same day she removed two more larvae which were taken to Dr. McClelland. The baby's throat when examined the following day showed from ten to twelve bright red spots covering an area of about three square inches. It is interesting that the comparatively slight amount of penetration of the skin by the larvae should have caused this marked irritation. We feel certain that the larvae had quite recently been deposited when the mother noticed them, otherwise they would not have been in a mass but would have wandered away or entered the skin.

Only one larva survived, measuring on June 25, 4 mm.; on June 26, 7–8 mm.; on June 27, 10 mm.; pupating on June 30 and emerging on July 13.

General Considerations of the Four Cases

In the four cases of myiasis in 1934, as well as in others previously reported from Toronto, the children had been sleeping out-of-doors. This fact emphasizes again the importance of keeping young babies carefully screened when sleeping or playing in the open.

Some significance may be attached to the observation that in two of the cases in 1934 and in one case in 1921 (Brady, 1923) the children were wards of Infants' Homes, having been placed in private homes where a small group of babies were being cared for by a foster-mother. It may be that the presence of several babies produces a more definite attraction for *W. vigil* and that in such homes special precautions by screening should be observed. On the other hand cases of myiasis are more likely to be reported to the medical authorities from these homes than by the average mother, since the women in charge are held particularly responsible for the babies in their care.

Myiasis Due to Wohlfahrtia vigil in Animals in 1934

During the summer of 1934 Dr. A. A. Kingscote of the Ontario Veterinary College had a number of cases of myiasis in animals brought to his attention from several places in Ontario. Dogs, cats, foxes, mink, ferrets and rabbits were infected. In his excellent paper (1934) he says, "A total of one hundred and eighty cases were definitely attributed to infection with the larvae of *Wohlfahrtia.* There is much evidence to indicate that this number is representative only of a small percentage of the cases which actually occurred." An analysis of cases, including records of other years, is summarized by Kingscote as follows: mink (149), dogs (20), cats (4), ferrets (4), rabbits (2) and fox (1). Kingscote says that "in very small animals from five to twenty larvae will cause death usually within about ten days."

Field Collecting in 1933

In the summer of 1933, on May 23, Dr. E. M. Walker took six specimens (all males) on the railway track at Donlands, a district north-east of Toronto. (Map 1, Station I.) Later on the same day the writer took 5 ♂ and 6 ♀. This was the beginning of a series of captures made throughout the summer. A number of trips were made to the district and flies were always seen, even as late as September 30th. In all, 62 males and 19 females were collected.

Several problems presented themselves. What is the attraction of the railway track, for the flies were at this time found nowhere else? Where do the flies normally deposit their larvae? What are their habits in the field?

From our earlier observations of the flies in captivity, we had expected to find them visiting various kinds of flowers, but this expectation was not fulfilled until the second and third years of collecting. In 1933, on May 23, when the apple trees were in full bloom, as well as the wild strawberries along the track, we searched for the flies on these plants, but none were to be found. Dead animals might have attracted the mature females, but a decomposing groundhog in the ditch was surrounded only by green bottles (*Lucilia*) and no wohlfahrtias were found here. A dead chicken lying below a railway bridge was also swarming with lucilias and other flesh-flies, but again wohlfahrtias were not present. On June 17 I hunted for the flies on the potentillas, buttercups, bladder-campions, yellow melilot, etc.; I examined the mouth of the burrows of the groundhog and the nesting-sites of the bank swallows, but only on the railroad tracks were the flies taken and they seemed to be most numerous in the vicinity of ponds. On July 12, of this same year, evidence was obtained that the flies visit the flowers of the milkweed, since individuals captured were carrying the pollinia.

Field Collecting in 1934

In the spring of 1934 we watched from early May at Donlands for wohlfahrtias. The tracks were visited also on May 23 and 24 and finally, on May 25 the first flies were seen. From this time on a few individuals were found whenever the district was visited, although the numbers were greatly reduced from that of the previous year. This reduction may have been due to the extreme cold of the winter. Temperatures ranging as low as −21.6° F. made this winter one of the coldest on record and as a result killed many plants and animals throughout the district.

An interesting record in 1934 was made as the result of investigating one of the cases of myiasis in an infant. The baby was living in an Infants' Home in a middle-class section of Toronto, the house being situated directly across the road from railway tracks and porcelain-ware

factories (Map 1, Case 4). On June 14, when the case was reported to us, we visited the district and made a careful examination of the railway track. The ground around the latter was so hot and dry that, as might be expected, no wohlfahrtias were found there. However, on following the track northward out of the city to Downsview, a point about two miles distant, we found a great number of the flies feeding on the flowers of a patch of wild caraway (*Carum Carui* L.) close beside the track (Map 1, Station IV). It is of interest that at this place there is a siding on which trains wait to pass each other on the single tracked road. It is very probable that flies alighting on the cars would be carried from here into the railway yards of the city.

The flies were local in their distribution, being found only on this one patch of caraway, although there were other clumps in both directions on the track. A collection of some twenty-five individuals was made and a hundred or more flies observed on the flowers. We examined the fields and highways nearby where buttercups, daisies, vetch, wild roses, potentilla, etc., were in bloom, but no wohlfahrtias were to be seen.

A few days later the railway employees cut down the wild caraway in their work of clearing the weeds. After that no further trips were made to the spot until August 1st and 30th, when no wohlfahrtias could be found.

Field Collecting in 1935

On all collecting trips around about Toronto and throughout the province, we have looked for wohlfahrtias and, as a result, four additional localities were added to the list in 1935. One of these was on the shore of Lake Huron at Baysfield, Ont., three were close to Toronto. Of the three latter localities the first was along the railway at a point a half a mile from Donlands (Map 1, Station III); the second beside the Humber River at Mount Dennis, a suburb of Toronto and about one mile from Downsview (Station V); the third out in the country three miles west of the Humber River on Queen Street, near Brown's Line Road and close to the Canadian Pacific Railway (Station VI).

Collecting during 1935 was more uncertain than in the two previous years. On June 6 we visited Donlands and captured two males in the usual place on the railway track. On June 9 Downsview was visited and again on the patch of wild caraway a few flies were feeding. One male was taken. But by July 3 and 4, when the wild caraway had gone to seed, there was not a wohlfahrtia to be seen at Downsview. On that occasion, we walked along the track in both directions, examining clumps of *Potentilla recta,* wild roses, etc., searched in nearby woods, beside small creeks and ponds, but no flies of the genus *Wohlfahrtia* could be

found. Apparently the flies leave this habitat as soon as the wild caraway fades.

On July 5, for the first time in three years, there were no wohlfahrtias to be found at Donlands. The day was very hot and the heat on the tracks almost unbearable. On July 18 three of the flies were seen on the tracks at Station III. Two days later we re-visited this locality and searched for an hour, from 1–2 P. M. Again the tracks were so hot that one could not keep one's hand on the metal rails. No flies were on the rails nor sleepers, and on the plants along the narrow path beside the rails only three flies of any kind were noted, these were captured and all were males of *Wohlfahrtia*. Two of the flies were carrying pollinia of the milkweed. Then for an hour a search was made among the milkweed, where flies of several kinds were feeding, but not *Wohlfahrtia*. The railway at this point has to the north of it spring-fed ponds and to the south runs a tributary of the Don River.

A new locality with no close association of railroad tracks was discovered on July 26, beside the Humber River at Mount Dennis (Station V). The first male fly taken, carrying a pollinium of milkweed, was resting on a path close to the water, the other three were feeding on wild carrot (*Daucus carota* L.). It was a fairly cool, sunny day, following two days of heavy showers and the wild carrot was swarming with many kinds of flies and bees, often ten or fifteen individuals on a flower head. Collecting was done from 2–3 o'clock, and not a female *Wohlfahrtia* was seen or taken. Some time was spent hunting for females in shady places, especially around the leaves of elecampane (*Inula helenium* L.) where other flies were hidden, but without success.

The next evening a search was made from 6.30 to 7.30 o'clock, with the sun occasionally shining through a low bank of clouds. At this late hour no flies of any kind were on the wild carrot, but all were now feeding on the white sweet clover (*Melilotus alba* Dest.). At 7 o'clock one large female of *Wohlfahrtia* was taken on the latter plant.

In July 29 three males were captured at the same locality between 2 and 3 o'clock, the first feeding on the clover, the other two on the carrot.

On August 2 the flies were found in a large field (Station VI) crowded with wild carrot and the early goldenrod (*Solidago juncea* Ait.). Collecting was done from 2–3 o'clock in a light drizzle of rain. Many flies of several kinds were feeding on the flowers and here and there were wohlfahrtias. In an hour's collecting seven males and six females were taken.

Evidence of an Association of Wohlfahrtia with Railway Tracks and with Water

It is noteworthy that so frequently *V. vigil* has been collected on tracks and, if not there, by water, the records being:

Port Sydney, Ont.	1919	N. K. Bigelow
R. R. Lake Abitibi, Ont	1925	E. M. Walker and N. K. Bigelow
R. R. Donlands, Ont. Stations I and II	1933–34–35	E. M. Walker and N. Ford
R. R. Downsview, Ont.	1934–35	E. M. Walker and N. Ford
R. R. Donlands, Ont. Station III	1935	N. Ford
Humber River, Mount Dennis, Ont.	1935	N. Ford
Brown's Line Road, Ill., Ont., near R. R.	1935	E. M. Walker and N. Ford
Lakeshore at Baysfield, Ont.	1935	E. M. Walker

It is also significant that in the cases of myiasis among children which we have investigated the baby in each instance was living close to a railway track. In the first case in 1934, as described above, the house was directly across the road from tracks (Map 1, Case 4). The second case occurred near the same railway as the first, the child living a short distance away in its own home (Map 1, Case 5).

In the third case the Infants' Home was situated in a good residential part of Toronto, directly across the road from a large natural park, where the street railway makes a terminal loop and three-quarters of a mile from three railways (Map 1, Case 6). In the fourth case the house was situated in the suburbs of New Toronto in a newly developed factory district operated by gasoline and oil companies, and was quite close to the tracks of the Canadian National Railway (Map 1, Case 7).

Finally as additional evidence of this association we would point out that in the case of myiasis which occurred in 1931 (Ford, 1932) the child, although living in one of the best and newest residential districts in Toronto, was less than a block away from a small railway cut which runs into the yards of a coal company (Map 1, Case 3). Also in the two cases in 1919 (Walker, 1920) the children were living close to tracks; one in the vicinity of Reservoir Park and along the Canadian Pacific Railway (Map 1, Case 1); the other in the west end of Toronto, the house crowded between two railway tracks and close to a third (Map 1, Case 2). We believe that the flies are brought into the city by trains.

The question of why the flies were taken so frequently on the railway track has been partly answered by the results of laboratory experiments in heat reactions.

Laboratory Experiments in Heat Reactions

To test the reaction of *Wohlfahrtia* to heat a multiple temperature incubator was used. When a fly was placed in this incubator with temperatures ranging from 24.6° C. to 31.8° C. the insect kept to the warmer end of the apparatus and settled down for the night at a point registering 28° C. The following morning (when the temperature of

MAP I. Map of the city of Toronto and vicinity showing the distribution of *Wohlfahrtia vigil* (Walk.) in relation to railways, streams and parks. Solid circles mark the stations where flies have been taken; open circles the location of the case of myiasis. Scale 0.35 inches to the mile.

the incubator had risen) the fly was resting at 33° C. and eggs had been laid on meat at a temperature between 27° and 29° C.

It was found during the cool days of September, when the room temperature was about 22° C. and when the flies were depositing neither eggs nor larvae from their distended bodies, that ovi- or larviposition could be induced by placing the flies at higher temperatures.

It would seem therefore that the warmth of the metal tracks has a definite attraction for the wohlfahrtias and this is the explanation which we offer at the present time for their presence there. Similar places of high temperature, such as exposed rocks or metal buildings, may in subsequent collecting also prove to be favorable resting-sites for the flies.

Reactions of the Female Wohlfahrtia to Light

The time of activity of the females in the field is unusual. On the early collecting trips in 1933 only males were taken, with the exception of May 23, when at sundown six females were captured. By June 17 of that year we had left alive twenty-seven males and one small female. This was discouraging from the standpoint of any experiments which had been planned. It was suspected that the females were probably resting quietly in some corner of the tracks, since they were not on the plants or flowers, and because in captivity the females hide around the edges of the cage.

On June 20 we commenced collecting after 5 o'clock and at first took only males. However, as the sun was setting at about 7 o'clock, we searched again. The males had now disappeared and in their places, resting very conspicuously on the shining rails, were the females.

On July 12 twelve males and five females were taken and again all but one of the females were captured late in the day, after 6.50 o'clock. September 30 was a very warm day for autumn and eight males and one female were collected, the latter being taken at sunset and in the region of the railway track where the shadows were lengthening.

In 1934 we again found that the female flies were rarely active during the afternoon. On the first day of collecting at Donlands, May 25, only males were taken. On June 14 collecting at Downsview at 2 P. M. and from 5–8 P. M. only males were seen until between 7 and 7.30 o'clock when three females were captured. These latter were resting on the rails in small sunny spots, while the greater part of the tracks were covered by the long shadows of late evening. By this time all the males had disappeared from the flowers and those which we had imprisoned in test tubes (and left lying in the grass) were asleep.

That the females are active in the earlier part of the day has been shown by Miss Blanche Snell who collected from the same patch of caraway at Downsview between 9 and 10 A. M. and at that time took four males and two females.

In 1935 a female was taken at Mount Dennis feeding on white sweet clover (*Melilotus alba* Desr.) at 7 o'clock in the evening on July 27. A few days later, August 2, the males and females were collected in approximately equal numbers (7 ♂, 6 ♀) feeding on flowers (*Daucus carota* L. and *Solidago juncea* Ait.) in a drizzle of rain, between 2 and 3 o'clock in the afternoon.

It would seem therefore that the female flies definitely avoid the bright sunshine. Further evidence of this tropism is found in the behaviour of the females when parasitizing a young mouse (see page 322).

In addition to the negative heliotropism of the females, their period of activity continues in the evening after the time when the males have become dormant. This marked difference in the behaviour of the sexes was well illustrated by the flies collected on August 2nd. They had been left in test tubes in the collecting kit until 7.30 P. M. when they were released one by one into a large cage. At this time the males were asleep in their test tubes and each of the seven tumbled stupidly upon the floor of the cage: on the other hand all six females were active, moving about in their tubes, and on being freed into the cage buzzed about energetically.

The activity of the females in dim light may well be a specialization associated with their parasitic habits of entering the burrows of mammals to parasitize the young. On the contrary, however, they are not known to enter houses, and all the children who have been parasitized were sleeping out-of-doors. Similarly the flies of the European species, *Wohlfahrtia magnifica,* "are flower feeders and never enter houses" (Patton, 1921–22), even though *W. magnifica* is parasitic upon the adults of both man and animals, as well as their young. It may well be claimed that a house bears little comparison with the burrow of an animal; the baby's carriage having a closer analogy. Moreover children sleeping in the open are usually left in a shady place, such as would attract *Wohlfahrtia.*

The following table summarizes the collecting records in the field. The smaller number of females is not due to any paucity of numbers, but to their lesser activity during the day. Reared in the laboratory the ratio of males and females is practically equal, as was shown by the first generation to emerge which consisted of 56 ♂ and 53 ♀.

Larviposition

Observed in the cages the females usually rest motionless and hidden in corners, although they sometimes come out in the sun. In this behaviour they contrast sharply with the males which are definitely heliotropic, gathering at the sunny end of the cage where they are active and quarrelsome. Guinea pigs, which were used as host animals, fortunately

COLLECTING RECORDS OF *Wohlfahrtia vigil*

1933			
May 23, Donlands	6♂		1–2 P.M.
	5♂	6♀	5–6 P.M.
May 27, Donlands	4♂		1–2 P.M.
June 14, Donlands	3♂		1–2 P.M.
June 17, Donlands	14♂		2–4 P.M.
June 20, Donlands	10♂		5–6 P.M.
		7♀	7–7.30 P.M.
July 12, Donlands	12♂	1♀	4–6 P.M.
		4♀	6.50–7.20 P.M.
Sept. 30, Donlands	8♂		4–5 P.M.
		1♀	5 P.M. (Sunset)
	62♂ and	19♀	
1934			
May 25, Donlands	3♂		1–2 P.M.
June 14, Downsview	6♂		2 P.M.
	11♂		5–7 P.M.
		3♀	7–7.30 P.M.
June 16, Downsview	2♂	4♀	9–10 A.M.
Aug. 27, Donlands	1♂		2 P.M.
	23♂ and	7♀	
1935			
June 6, Donlands	2♂		1–2 P.M.
June 9, Downsview	1♂		2–3 P.M.
July 20, Don Mills Road	3♂		1–2 P.M.
July 26, Humber River, Mount Dennis	3♂		2–3 P.M.
July 27, Humber River, Mount Dennis		1♀	7 P.M.
July 29, Humber River, Mount Dennis	3♂		2–3 P.M.
Aug. 2, Brown's Line Road, Ill.	7♂	6♀	2–3 P.M. (in drizzling rain)
Aug. 16, Baysfield, Ont	1♂		2–3 P.M.
	20♂ and	7♀	

Grand Total: 105♂ and 33♀

prefer the shadows, and it was therefore very evident when a female became interested in the mammal. As she followed the guinea pig to the shady end, she was separated from the other flies, and undisturbed she would exhibit the characteristic behaviour reactions of sitting and watching the guinea pig, or else of becoming excited and running in circles under the animal's chin.

One can tell when the flies are about to deposit larvae, the first signs being the nervous hurried movements, and the final one the position of the body, i.e., when the head is kept down and the abdomen held high in the air. At this stage the fly, from time to time, makes a sudden strike

at the head of the guinea pig, when many times it is promptly shaken off. In our previous work a fly after such a flight larviposited close to the eye of the guinea pig.

In 1933 the more interesting results were concerned with newly-born mice and rats. Such young animals are very active, rolling about, kicking and tumbling. However, when the flies are ready to deposit their larvae, they are most persistent and not easily disturbed. On May 27, 1933 young rabbits lying in a small box were placed in the cage. Two flies entered the box and stood cleaning their legs and wings, quite undisturbed by the movements of the rabbits. One fly would then alight on the head of the rabbit and hold on in spite of much twisting and turning. The rabbits were bumping the sides of the cardboard box, but the flies remained on the edge watching it all or else pacing up and down along the edge of the box.

On June 26 a young mouse, 5 days old, was placed in the cage. A female fly soon discovered it, ran about the animal, spending much time close to its face, examining particularly its mouth which was resting on the bottom of the dish. Suddenly the fly turned and pushed its abdomen close to the mouth and larvae were discharged. The fly changed to another position and more larvae passed out; finally from a third angle a number of larvae again left the body. Some twenty or thirty were deposited altogether. The little mouse lay with its mouth resting in the pile of larvae and when it finally raised its head, the mouth and chin were bristling with the hanging maggots.

Irritated, the mouse twisted about and as a result the majority of the larvae were brushed off against the side of the dish. Some larvae appeared to be entering the mouth. One vigorous larva, quickly crawling toward the mouth, covered a distance of one centimetre in a few seconds, and then as the mouse turned about, the larva crawled into a fold near the foot. The cephalo-pharyangeal sclerites, showing through the transparent cuticle of the newly-born larvae, gave the appearance of black heads which were raised perpendicularly above the ground, so that the larvae moved along like so many miniature ducks. Eggs as well as larvae were deposited. This suggests that the fly, overly-stimulated by the presence of a young animal, may have oviposited prematurely.

Before the mouse was removed from the cage a second fly deposited larvae on the foreleg. These larvae were even more active than the former ones.

The mouse was placed in a nest of cotton batting and left all night, warmed by an electric light bulb. The next morning the mouse was still alive with one larva embedded in its ear, another in the skin of the abdomen. Both wounds were inflamed and bloody. We returned the mouse to its mother, who accepted it, washed its sore ear, cleaned it and fed it.

By the next day we were surprised to find no sign of the larvae, the mother having apparently destroyed them. The mouse grew up as strong and healthy as his brothers.

On August 4 a new-born rat was put in the cage and a wohlfahrtia laid a mixture of fifteen or more larvae and eggs, mostly eggs. These also were placed by the fly in a mass in front of the rat's nose. Again it seemed that the presence of a newly-born animal induced a premature laying of eggs as well as larvae. The animal was kept warm under an electric-light bulb all night, but unfortunately there was no sign of larvae next day, a fact which supports the view that they were prematurely deposited.

On July 27, 1934 while working with my hand inside a cage, a female alighted on my arm and deposited six larvae. As had been previously observed the larvae are unable to penetrate the skin of an adult. On this occasion the posterior part of the body of the larva was used as a sucker and holding on with this each larva raised itself erect and then twisted its body into a V-shape as it scraped at the skin.

At times several females in a cage will pursue a young animal, as is shown in Fig. 2. In this instance one fly has crawled up on the head of the animal, while two others are waiting an opportunity.

In 1934 we had a second case of cutaneous myiasis in a mouse and in this instance observed the treatment which its mother gave it in removing the parasites.

Parental Care Given to a Parasitized Mouse

In July 1934 a young mouse, 5 days old, became parasitized while living with its parent in a darkened cage. The mouse cage (11 by 11 by 15 inches, made of sheet tin) had been placed in a large glass cage (18 by 28 by 38 inches) on July 26th. The mouse cage was closed by a round lid, 10 inches in diameter, in the centre of which we had cut a small hole, 1 inch in diameter.

Three female wohlfahrtias were put in the large cage. Two individuals were indifferent, but one was much interested. The latter alighted on the top of the mouse-cage, ran around the edge of the tin lid, apparently getting the scent of the mouse, then flew over the lid and in small circles above the hole. She then alighted, investigated the hole and crawled in a short distance. Soon she came out again and repeated the performance several times.

The following day interest was again evident in the fly. Two more female wohlfahrtias were added to the cage. So long as the cage was in subdued light the flies were active, but if a stream of sunshine was allowed to strike them all activity ceased. This reaction to light was especially marked on July 29th, the flies being active only when the blinds in the room were kept drawn.

In the subdued light one female kept walking around the lid of the mouse's cage, close to the narrow slit where the lid fitted the top. A piece of glass had been placed over the hole in the lid. This we now carefully pushed aside with a long wire, so as not to disturb the wohlfahrtia. The fly continued to encircle the lid and then to run around the displaced glass cover (showing an association with the smell of the mouse). In four minutes she discovered the uncovered hole, entered and went down into its darkness. An hour later she had failed to re-appear, so the hole was covered with the glass top. (Time, 4.30 P. M.)

The following day the mouse's cage was carefully examined but there was no sign of the fly. Did the mother kill the fly and eat it or was its body lost in the sawdust?

The young mouse had a lesion, 5 mm. in diameter, to the right of the tail, containing a number of larvae. The parental care given by the mother was most interesting. Almost continuously she worked with the little mouse (Fig. 3), washing the lesion or at times biting it, now holding the mouse down on its side as she worked or bringing it back into the nest when it frequently wandered away. She also fed it and washed its body.

Twenty-four hours later the lesion was much larger. Only one larva remained, which the mother had been unable to remove, and it was almost full grown. A second lesion now showed on the mouse's back, at a point close to the head of the larva (Fig. 4). The mother was tearing the skin at this point in an attempt to get at the parasite. The larva began moving about under the skin, thus crawling over to the left side. The mother was beside herself with excitement and fatigue. The little mouse was very sick and so it seemed best to chloroform and preserve it. When the body had been in formalin for 10 minutes or so, the larva crawled out from the lesion. The larva was washed, placed over sawdust, where it pupated two days later.

There were some doubts in our mind regarding the apparent rapid growth of the larva. It appeared that the larval developmental period was only four days: the larva being full grown in two days and pupating on the fourth day. Either body temperatures greatly hastened the growth rate or else the wohlfahrtia had previously deposited larvae on the lid of the cage and these had made their way down to the mouse. To clear this point, larvae were reared at high temperatures in the multiple temperature incubator.

Growth of Larvae at High Temperature in Incubator

On September 14, 1934 a female fly was placed in the multiple temperature incubator with temperatures ranging from 28.8°–38.2° C. The following morning the fly was resting at a point registering 33° C. and

larvae, as well as two clumps of darkened eggs (i.e. eggs about to hatch), had been deposited in a test tube at 34° C. The larvae were removed to the cooler end of the incubator. Here within 24 hours they grew to a length of 8–10 mm. This was a remarkably rapid growth, especially when compared with larvae kept at room temperature, 24° C., which had attained a length of only 4–5 mm.

The next day, September 17, the larvae in the incubator were separated and put in 9 test tubes, with 4 individuals in each tube and these were arranged along the incubator. On September 18 all larvae were thriving, even those at the highest temperature. On September 19 some of the larvae entered the sawdust; on September 21 some had pupated.

On September 23 the temperatures in the incubator ranged from 29.4°–40.6° C. In the test tubes there were the following larvae and puparia.

Test tube	Temperature centigrade	Larvae	Puparia
I	48 – 42	3, dead	1 shrivelled
II	42 – 38		3 normal 1 shrivelled
III	38 – 35		4
IV	35 – 34		4
V	33 – 32.5		4
VI	32 – 31.5		4
VII	31 – 30.5		4
VIII	30.5 – 30		4
IX	30 – 29.5	1	4

On September 29 two females emerged from test tube IV and on October 1 one male from t.t. VII and one female from t.t. VIII. (Other emergences followed but records are incomplete.)

The larval developmental period (September 15–20 and 22) was therefore 5 to 7 days; the pupal period (September 21 and 23–September 29 and October 1) was 8 days. Both periods were hastened when compared with the length of development at average July temperatures, i.e. 7 to 9 days for the larva and 10 to 12 days for the pupa. The rapid development at these higher temperatures approaches that of the parasite of the mouse and thus leads us to believe that the observation of four days for larval development in the mouse was quite accurate.

Care of the Flies in the Laboratory

The adults, in the laboratory, are kept in cages, 17 by 17 by 26 inches, made by covering coarse-meshed square waste-paper baskets with cheesecloth and painting them with melted paraffin around the edges where there are joins in the cloth. These make very substantial cages. Less expensive and less durable are cages made with a wooden framework and covered similarly with cloth. The cages are placed so that there will be both sun and shadow available for the flies.

The staple diet of the flies was water, milk, lump sugar and, in addition, fruit, such as cantaloupe, and flowers suitable for short-tongued flies.

To receive the larvae a fresh supply of rabbit muscle was kept in small Petri dishes in the cages. This sort of meat was used because it proved difficult to obtain unblown meat from the stores. A rabbit was killed, put at once into the frigidaire and then used as required. The Petri dishes in the cages were protected by a sheet of glass over the top of the cage, so that other flesh-flies outside the cage could drop neither larvae nor eggs upon the meat.

When larvae had been deposited (usually from 15 to 50), the Petri dishes were removed, securely covered and placed in a darkened cupboard. With the growth of the larvae additional meat was added and finally the whole mass was transferred to a quart sealer half filled with damp sawdust. A supply of fairly fresh meat must be available for the larvae, for if their food becomes very stale or rotten the larvae do not relish it and will be found wandering away and climbing the sides of the container.

Because of their feeding habits, it is very desirable to leave the larvae together, particularly while they are small. If separated a high mortality results. Brown (1936) in his account of the rearing of *Wohlfahrtia vigil* says: "Growth and feeding do not get under way for 1 or 2 days, awaiting probably a sufficient accumulation of ammonia for liquefaction of the meat. This is borne out by the fact that the young larvae normally congregate in an unbroken rank to attack the muscle; if separated they make no headway, and soon die." In human myiasis, as well as in animals, several larvae are often in one lesion. From time to time the larvae leave a lesion, wander over the skin and enter another pocket.

In rearing the larvae we had the experience on more than one occasion of all the individuals in a jar (60 or more) dying when full-grown. The sawdust about the larvae was stained deep yellow and we suspected the presence of ammonia. A similar stain was obtained by treating the sawdust with this chemical. Brown (1936) determined that for *Wohlfahrtia vigil* the "maximum ammonia excretion occurs approximately at the peak of the body-weight curve, when ingestion and excretion are equal in weight, which is just before the larvae leave the food," and that "ammonia excretion then falls off suddenly, finally to cease at pupation." It would appear therefore that the ammoniation in the prepupal period may at times be lethal to the larvae.

Roberts (1932) also refers to the production of ammonia in the larvae of *Phormia* and *Lucilia*. He says that when these larvae are used in maggot therapy "one of the first effects to be noticed in the wound is a change from an acid to an alkaline reaction, presumably due

to the vigorous production of ammonia which accompanies the liquefaction of meat by the larvae." Large amounts of ammonia are present in the excreta of *Lucilia sericata* (Hobson, 1931) and may be deposited in some quantity before pupation when the larvae decreases in weight. Stewart (1934) found in *Lucilia sericata* that "occasionally high mortality of the young larvae placed in such wounds (i.e. osteomyelitis wounds which had been treated for some time with blow-fly larvae) was due to ammoniation of the wounds by larval activity."

If the pupae are to be delayed in their development, they are gradually chilled and then placed in a refrigerator at about 11–14° C. Here they may be left or else transferred to lower temperatures. For six months we have successfully stored them, provided the sawdust is not allowed to dry out. How long it may be possible to store them we have not ascertained but on August 24, 1934, puparia were examined which had been in the frigidaire (temperatures, 6–10° C.) since August 2, 1933, i.e., for over a year. Although some of the insects had died and disintegrated, others were living. The latter failed, however, to emerge in the laboratory, but this may have been due to several unfortunate changes of the temperature during the periodic breakdown of our automatically controlled cooling system.

When the adults are emerging special precautions must be taken to prevent their escape, since before the wings are blown and while the frontal sac or ptilinum is inflating the flies can push their way through several layers of cheese-cloth. The flies are particularly likely to attempt this if they are in close quarters, but in a large cage there is not the same danger. It has become our custom to place the uncovered quart jar containing the puparia in a large cage with a narrow strip of cheesecloth hanging over the edge of the mouth of the jar, touching both the sawdust and the bottom of the cage. This strip serves as a "ladder" by which the flies can quickly and easily leave the jar and settle down in the cage until their cuticula hardens.

Length of the Life Cycle

The adult life span of a single individual of *Wohlfahrtia* was carefully recorded in 1931. At that time the fly mated three days after emergence and larviposited on the eleventh day. A second mating occurred on the 41st day and larvae were deposited on the 48th and 49th days, the fly dying on the 53rd day.

In 1934, when many flies were kept in each cage, the records were more general. During the summer months, larviposition took place from 13 to 17 days after emergence and the flies lived from 30 to 40 days. The developmental period of the larvae at July temperatures was 7 to 9

days; the pupation period from 10 to 12 days; the life-cycle being completed in from 30 to 36 days.

When kept together in a cage, the males die much earlier than the females. For example, 5 males collected on June 14, 1934, died on June 15, 16 and July 3; while 4 females taken at the same time lived until July 6, 10, 11 and 17. Thus all the males were dead in 19 days, but the first female did not die until the 22nd day and the last on the 33rd day.

If the males are kept by themselves they have a longer span of life. Individuals taken on May 25, 1934, lived for 29, 37 and 43 days isolated.

Acknowledgments

I wish to give much credit to Dr. E. M. Walker for his unerring instinct and keen eye in discovering the adults of *Wohlfahrtia vigil* in their natural habitat, where they have been so rarely taken, and to thank him for his generosity in sharing this information, and for his interest in the whole problem under investigation. I should like also to thank Dr. Alan Brown and Dr. L. N. Silverthorne of the Sick Children's Hospital, Toronto, as well as Dr. J. H. McCelland and Dr. S. G. Parker, for the opportunity they gave us of studying the several cases of human myiasis; to express my appreciation to Dr. A. A. Kingscote for his co-operation in discussing with us the many cases of animal myiasis which came under his observation and the problems of control which they involved; to thank Mr. A. W. A. Brown, who undertook the determination of the excretion of ammonia and uric acid during the larval period; to thank Dr. R. D. Defries and Dr. J. Craigie, Connaught Laboratories, and Mr. J. W. Ansley, York Memorial Collegiate, for the loan of ciné equipment; to thank Mr. F. Athron, Eastman Kodak Company, for helpful direction in photography; Miss Blanche Snell for assistance in collecting; Miss Marjory Ford for rearing material; and Mr. W. LeRay and his assistants for animal material and aid in conducting experiments.

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PATHOLOGICAL STUDIES ON THE PENETRATION OF THE CERCARIA OF THE STRIGEID TREMATODE, *DIPLOSTOMUM FLEXICAUDUM*[1]

DORLAND J. DAVIS

INTRODUCTION

It is the purpose of this paper to report some observations and experiments conducted during the summer of 1935 at the University of Michigan Biological Station on the penetration of the cercaria of *Diplostomum flexicaudum* (Cort and Brooks) into abnormal hosts. Van Haitsma (1930) determined the life history of this strigeid as follows: The adult form inhabits the intestinal tract of the herring gull, *Larus argentatus smithsonianus*. Eggs in the feces are deposited in water, and from these miracidia hatch in about three weeks. The miracidia penetrate the snail, *Stagnicola emarginata angulata* (Sowerby), and there develop into mother sporocysts which in turn produce daughter sporocysts. After about six weeks cercariae are developed within the daughter sporocysts and escape from the molluscan host to swim about freely in the water. They penetrate the body of the common sucker, *Catastomus commersonii,* and lodge in the lens of the eye where they metamorphose into the diplostomulum stage in about six weeks. By eating the fish the gull becomes infected, and the worm reaches sexual maturity in its intestine in less than a week.

Although the common sucker is the only fish with which the complete development of the metacercaria stage and infection of the definitive host with this species has been obtained, these cercariae will penetrate into a number of different fish and amphibians. Cort and Brooks (1928) in connection with the original description of the cercaria of this species stated that it was found to penetrate actively into several different species of fish and that it developed into a metacercaria of the diplostomulum type in the lens of the eye. La Rue, Butler and Berkhout (1926) reported the finding of strigeid metacercariae of the diplostomulum type in the eyes of several different species of fish from Douglas Lake, Michigan. They were most common in the eyes of *Catostomus commersonii, Perca flavescens,* and *Percopis omiscomaycus* but were also found in

[1] This paper is a contribution from the University of Michigan Biological Station and the Department of Helminthology of the School of Hygiene and Public Health of the Johns Hopkins University.

The author wishes to express his grateful appreciation to Professor W. W. Cort, under whose guidance this work was carried out. Thanks are also due to Dr. L. J. Thomas of the University of Illinois for helpful suggestions and to Dr. G. F. Otto of the School of Hygiene and Public Health of the Johns Hopkins University for assistance with the illustrations.

Ambloplites rupestris, Ameiurus nebulosus, Lepomis gibbosus, Lota maculosa, and *Notropis cornutus.* It is evident that all their findings probably do not represent metacercaria stages of *D. flexicaudum* since there are other strigeid cercariae in Douglas Lake that penetrate into fish and at least one other species, *Cercaria laruei* Cort and Brooks, in which localization is known to be in the lens of the eye.[2] Dr. Cort's unpublished records further show that the cercariae of *D. flexicaudum* will penetrate into several species of fish, besides *Catostomus commersonii* which has been shown to be a normal second intermediate host. These include *Notropis hudsonius, Perca flavescens, Salvelinus fountinalis, Ameiurus nebulosus,* and *Percopsis omiscomaycus.* Whether these fish can serve as normal second intermediate hosts for *D. flexicaudum* is not at present known. There are records also of penetration into tadpoles, where the larvae reach the eye but fail to develop normally.

Van Haitsma (1930) observed that the penetration of large numbers of the cercariae of *D. flexicaudum* into fishes caused definite pathological lesions and death in many instances. He suggested this as a cause of death of large numbers of fish in our small lakes every year. In the unpublished records discussed above it is also noted that large numbers of the cercariae commonly killed small fish of the species into which they penetrated. Blochman (1910) observed that the furcocercous cercariae, *Cercaria fissicauda* La Val., were lethal to fish placed in an aquarium containing infested snails. He concluded that the actual means of producing death were the movements of the cercariae through the tissues especially through the vascular and central nervous system and was not the result of a toxin produced by the cercariae. Cort (unpublished) further noticed that the cercariae of *D. flexicaudum* would penetrate frog tadpoles and kill them in relatively small numbers.

It is evident from the above discussion that the cercariae of *D. flexicaudum* will penetrate into a number of species of fish and produce injury and death when the numbers are large. In Douglas Lake and the other lakes in the northern part of the southern peninsula of Michigan, *Stagnicola emarginata angulata* which is the commonest host of the cercaria of *D. flexicaudum* is a very common and widely distributed beach snail. How common the cercaria of *D. flexicaudum* is in this host in this region is shown by the records of its incidence given by Cort, McMullen and Brackett (In press) for collections of *Stagnicola emarginata angulata* made during the summer of 1935. They found in 2,526 specimens of *S. emarginata angulata* collected from three localities a total of 1,155 infested with the cercaria of *D. flexicaudum* or 42 per cent. This extra-

[2] I wish to express my appreciation to Dr. W. W. Cort for the use of this and the other unpublished records that follow from observations which have been made over a period of several years at the University of Michigan Biological Station.

ordinarily high incidence of this species of cercaria has been found in this general region wherever collections of the snail host have been made. In fact in most collections of this snail the incidence of this species is greater than that of all the other species of cercariae present. Such findings certainly support the suggestion of Van Haitsma that the cercaria of *D. flexicaudum* must be an important factor in the killing of young fish.

At Professor Cort's suggestions the writer undertook to study the pathological effect grossly and microscopically of the penetration of the cercaria of *D. flexicaudum* into frog and toad tadpoles. These cercariae will penetrate into these animals and work their way through the body toward the eye producing injury by their migrations and death when present in any considerable numbers.

PENETRATION

The actual mechanism of the entrance of all cercariae into the body of vertebrates is not clearly understood. This species is equipped with glands which have been supposed to secrete a substance which aids in their penetration. Experiments of the writer (Davis, 1936) showed that an aqueous extract of macerated cercariae of *D. flexicaudum* contained material which has a digestive effect on tadpole skin and muscles. It seems clear, therefore, that a histolytic ferment is secreted from glands in the bodies of the cercariae of this species, which digests host tissue and permits the entrance of the larvae. In addition they have a clump of forward pointing spines on the anterior tip and backward pointing pines about the oral sucker to aid them in penetrating the skin of the host and in working their way through its tissues.

Microscopic examination of sections 20 μ in thickness stained with Delafield's hematoxylin and eosin showed cercariae pushing their way through the integument of the tail fin of a tadpole. A photomicrograph (fig. 1) shows that epidermal cells are pushed aside slightly, and a few of them are disintegrated. The basement membrane is pierced, and the stroma margin is pushed back a little. A mass of leukocytic cells is gathered about the anterior end of the invading larva as though to repel it. Scattered here and there are a few nucleated red blood cells.

Several different animals were exposed to these cercariae in an effort to determine which ones could resist the invasion. The tadpole stage of *Bufo americanus, Rana clamitans,* and *Rana pipiens* were found especially susceptible to them and were killed in a short while. Individual tadpoles were placed in two inch stender dishes containing various numbers of cercariae. It was found that the tadpole of *B. americanus* was killed in three hours when approximately three hundred cercariae were put in the dish with it. The larger tadpoles of *R. clamitans* and *R. pipiens* required twice as many cercariae in the same length of time to

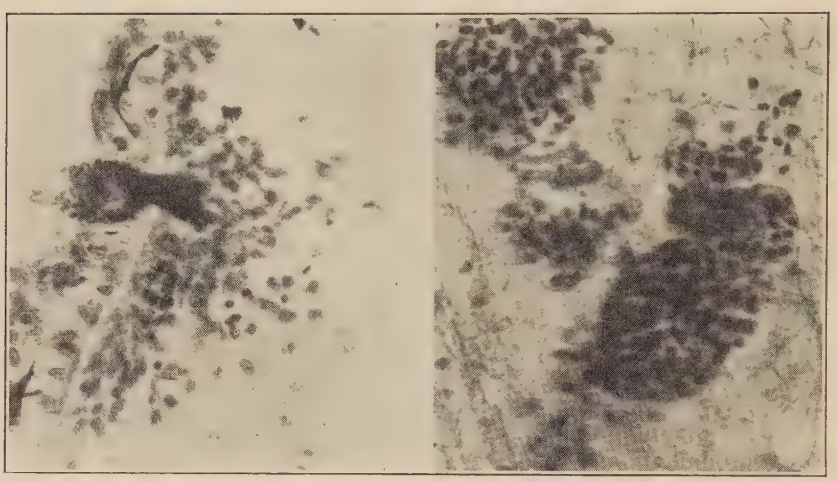

FIG. 1 (left). Photomicrograph magnified about 400 times showing a cercaria penetrating the integument of a tadpole. The epidermal cells are pushed aside slightly, a few being disintegrated; the basement membrane is pierced, and a mass of leukocytic cells is gathered about the anterior end of the invading larva as though to repel it. A few nucleated red blood cells are present.

FIG. 2 (right). Photomicrograph magnified about 400 times showing extravasated nucleated red blood cells in channels in the stroma where the fibrous tissue has been broken by the migration of the metacercariae.

kill them. A hundred cercariae were usually lethal to a toad tadpole in twenty-four hours, but if it survived for that period of time it did not die. Small bullheads, *Ameiurus nebulosus,* three to four centimeters long were even less resistant than either of the amphibians succumbing in three hours to one hundred cercariae. Young *Amia calva* and adult *Triturus viridescens* were resistant and survived large numbers of cercariae although a few larvae did penetrate into the young *Amia calva.* The method of estimating the number of cercariae used in these experiments was to place a few drops of water containing them in the bottom of a dry stender dish and count the swimming forms with a dissecting microscope. This was quite possible up to a hundred or so. To estimate more than that, an even suspension of cercariae was made in a stender dish and the average number of cercariae per drop was determined, and then the required number of drops added to a dish. While this necessarily did not give an accurate count, it was a good approximation.

Small recently metamorphosed frogs weighing within a few tenths of a gram of the large tadpoles that succumbed were not affected by the cercariae though exposed to large numbers over a long period of time. Small toads that had metamorphosed a day or so previously in the laboratory and weighed less than half a gram were also resistant to penetration. The skin was removed from a leg of each of five small frogs and

the legs exposed to several hundred cercariae, an infection which they had withstood before the skin was removed. In a few hours they were dead and the leg showed petechial hemorrhages. Metacercariae were found in the brains and eyes of all. One was found in the lung of one of the frogs and in another of the frogs the gall bladder was ruptured. It is evident that some change occurs in the skin at metamorphosis which renders it immune to penetration. The two principal changes which take place in the skin of frogs during metamorphosis (Gaupp, 1904) are a thickening by increase in the number of layers and the appearance of mucous and poison glands. The skin, instead of a simple epidermis two or three cells in thickness, becomes a complex integument having three distinct layers—the epidermis, the corium containing the glands and made up chiefly of connective tissue with some smooth muscle, and the tela subcutanea which is next to the lymph sack. Either this integumentary barrier is too thick and horny for the cercariae to pierce or the mucous and poison gland secretions kill or inactivate them.

REACTION OF THE HOST

Tadpoles exposed to the cercariae usually showed increase in activity for the first half hour and often jumped out of the dish from the irritation. Soon tiny hemorrhagic spots appeared on the body and they became less active. Half an hour before death they became very sluggish and often turned over on their backs moving their tails very feebly. The gill movements were last to cease.

On autopsy the tail and body showed many hemorrhagic spots where the cercariae had penetrated, and a hemorrhagic ring was conspicuously present about each eye. The anterior thoracic area showed hemorrhage through the semi-transparent skin, which was confirmed on microscopic examination, as though the great vessels of the aortic area had been ruptured. Often free blood was found in the body cavity. This internal hemorrhage could easily account for their death. Many metacercariae were discovered alive and moving in the brain, as well as in the eye and lens of the eye, and were frequently seen in the lumen of the heart, in the liver, gills, kidney, and intestinal tract.

Sections were made of certain of the tadpoles which had been killed by the cercariae to study the histological reactions. Penetration has been described previously in this discussion. The host offers little resistance to this invasion, though it is probable that a few larvae may be destroyed by the leukocytes of the tadpole. An occasional metacercarial form was seen in the stroma surrounded by leukocytes which were evidently attempting to destroy and wall it off. However, most metacercariae seen in the tissue had only an occasional leukocyte near them and were probably actively penetrating. There was considerable hemorrhage, and the

path of the larvae could be followed by the extravasated nucleated red cells in channels in the stroma where the fibrous tissue had been broken (fig. 2).

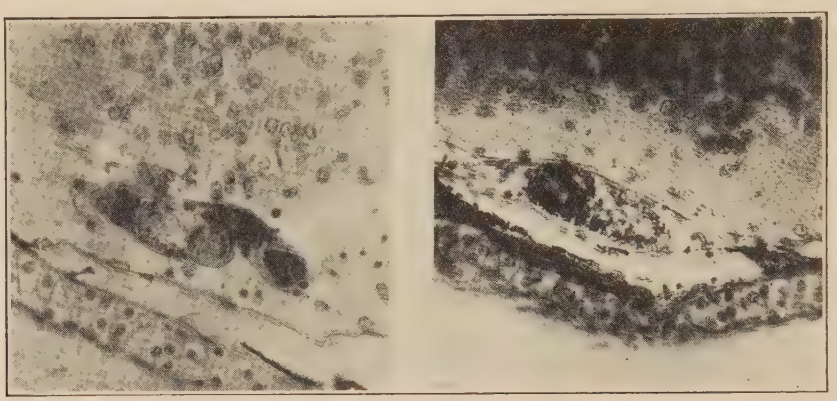

FIG. 3 (left). Photomicrograph magnified about 400 times showing a metacercaria between the brain tissue and the chondrocranium. The suckers can be plainly seen.

FIG. 4 (right). Photomicrograph magnified about 400 times showing a metacercaria located between the meninges of the brain.

MIGRATION IN THE BLOOD STREAM

The next problem to be considered was just how the invading cercariae got from the point of entrance to various parts of the body. Two courses are open: They can either plow their way through the tissue directly to the final destination, or they can make their way into a blood vessel and be carried there in the blood stream. While a good many do plow through the tissues, it is the opinion of the writer that most find their way into blood vessels and are carried to distant parts of the body. Several experiments to show this were performed. Two frog tadpoles were held for ten minutes with the tips of their tails in water containing a heavy concentration of cercariae. They died in about three hours and on autopsy two metacercariae were found in the brain of one and two in the eye of the other. Since the only place they could have penetrated was the tail they had traveled to the head in two to three hours. This seems too rapid for direct penetration of tissues and suggests that they were carried by the blood stream. Experiments cited previously with the skin stripped from the frog leg show the same results. Another observation was made in this way: A frog's tongue was pinned out over a hole in a piece of cardboard and placed under the microscope in the same manner that capillary circulation is studied. The blood vessels are easily visible. A drop of water containing many cercariae was then placed on the tongue and penetration watched. The cercariae were seen

to wriggle about, attach themselves and lose their tails, and then by undulating movements try to work their way into the skin. In ten or fifteen minutes they could be seen under the mucous membrane moving through the tissues. In a few more minutes several could be observed to pierce the walls and enter the lumen of the arteries and veins. By means of their suckers they traveled along the inner wall of the blood vessels. Some were carried along for a short distance by the stream, then attaching themselves to the wall would inch along for a space and then be washed off and carried on. The metacercariae would usually try to move against the current and could do it fairly well for a little while. In a microscopic section of the tail a metacercaria was seen occupying nearly the entire lumen of one of the veins. Sections of the brain showed them most frequently in the subchondral space and between the meninges which have a rich blood supply (figs. 3 and 4). This evidence seems to indicate that an important mode of migration of these cercariae is by the blood stream.

FINAL LOCATION OF METACERCARIAE

At autopsy the metacercariae were most easily and frequently found in the brain and the eye. One explanation for this is that the head region receives a large blood supply and the cercariae carried by the blood are caught in the arteries then plow their way out into the tissues of the eye, brain, and cord. It seems probable, however, that there is some attraction on the part of certain tissues for these particular forms since the lens of the eye is the normal site of the location of the metacercariae. In order to test whether there is such a histotropic effect the following experiment was undertaken: Various tissues from tadpoles were removed and placed in stender dishes containing cercariae. After half an hour they were examined microscopically in a water preparation under a cover slip. To get an idea of the relative numbers of cercariae in each tissue they were counted in ten low power fields. Fifty such fields for each tissue, five samples being used, were examined and 116 cercariae were counted and found in the following places: brain, 38; muscle with skin attached, 29; eye, 27; liver, 11; muscle, 10, and heart, 1. This seems to indicate that certain tissues, that is brain, muscle with skin attached, and eye, had offered better conditions for them than other situations. Studies of serial sections indicate that these larval forms are found in these same sites most commonly (figs. 5 and 6).

When these metacercariae lodge in the brain one would think that the trauma would be likely to cause some sort of central nervous system symptoms. A toad tadpole which was exposed to 200 cercariae on June 26 in a few hours showed hemorrhagic spots and became sluggish. Next morning it was floating motionless on its back but had regular gill and

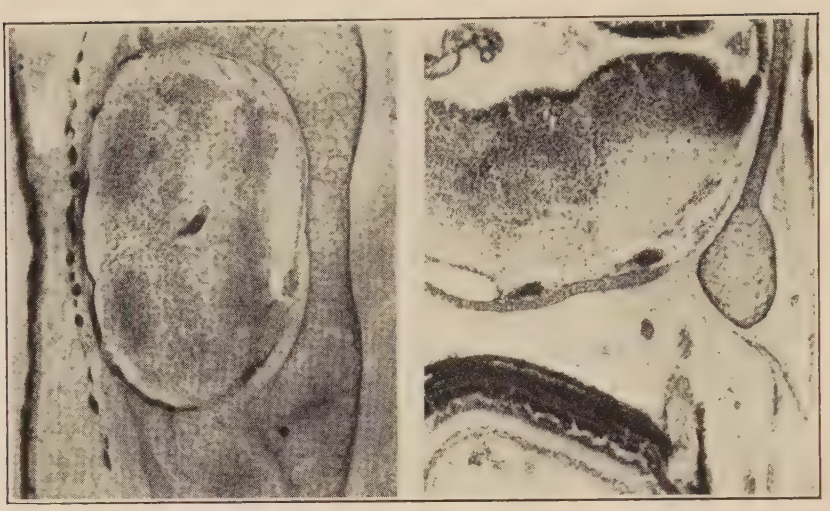

FIG. 5 (left). Photomicrograph magnified about 100 times of a section of the brain stem with a metacercaria in the middle of the brain substance.

FIG. 6 (right). Photomicrograph magnified about 100 times of a section through the brain and eye showing two metacercariae located between the brain tissue and the chondrocranium.

mouth movements. Through the dissecting microscope its heart was seen to be beating. There were a few head movements but none in the body. Each morning for the succeeding ten days it showed only gill and mouth movements, and the heart continued to beat. No peristalsis or evacuation of feces could be detected as in other specimens. It became very emaciated and died on the eleventh day. The path of the invading organism through the nerve centers and tracts had obviously caused sufficient injury to produce this apparent paralysis.

SUMMARY

Experiments and observations here cited justify the following conclusions:

1. The cercariae of *Diplostomum flexicaudum* penetrate directly through the epidermis of tadpoles and in sufficient numbers will kill them.

2. The change in the skin which occurs at the metamorphosis of frogs and toads renders them immune to the penetration of these cercariae.

3. The host attempts to resist the invasion by an outpouring of leukocytes but is not successful in most instances.

4. Cercariae of this species may be carried to distant parts of the body by the blood stream.

5. These metacercariae tend to lodge most commonly in the eye and in nervous tissue.

6. Injury to the central nervous system may cause paralysis, and injury to the vascular system brings about fatal hemorrhage.

Literature Cited

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A CATALOG AND HOST–INDEX OF THE GENUS *PLASMODIUM**

G. Robert Coatney and Robert L. Roudabush

In an effort to compile a catalog and host-index of the genus *Plasmodium* the authors have made a sincere effort to examine the original literature, but in a few cases, as indicated, it has been found necessary to accept the statements of other workers who, it is hoped, had access to the original descriptions or host lists.

The species are numbered consecutively and the corresponding number or numbers placed preceding the author's name in the host-index, providing the author indicated the species found. Certain species, originally placed in the genus *Plasmodium* but which for evident reasons belong in other genera, have been retained in the catalog and footnotes are appended to indicate the correct genus. No decisions are advanced concerning synonymy. The catalogers invite correspondence concerning errors or omissions.

Genus Plasmodium Marchiafava and Celli, 1885

Synonyms: *Laverania* Grassi and Feletti, 1890, *partim*; *Haemomoeba* Grassi and Feletti, 1890 *partim*; *Cytosporon* Danilewsky, 1891, *partim*; *Proteosoma* Labbé, 1894.

Catalog of Species

1. *P. achromaticum* Yakimoff and Stolĭnikoff, 1912. In *Achromaticus vesperuginis,* bat.
2. *P. agamae* (Wenyon, 1909). In *Agama colonorum,* lizard.
3. *P. biziurae* (Gilruth, Sweet and Dodd, 1910). In *Biziura lobata,* musk-duck.
4. *P. bouilliezi* Leger, 1922. In *Cercopithecus campbelli,* monkey.
5. *P. brasilianum* Gonder and von Berenberg-Gossler, 1908. In *Cacajoa* (=*Brachyurus*) *calvus,* monkey.
6. *P. brasilianum* Gonder and von Gossler, 1909. In *Brachycerus calvus,* monkey.
7. *P. brodeni* Rodhain, Pons, Vandenbranden and Bequaert, 1913. In *Petrodromus tetradactylus,* jumping rat.
8. *P. bubalis* Sheather, 1919. In *Bubalis* sp., plains buffalo.
9. *P. camarense* Ziemann, 1915. In *Homo sapiens,* man.
10. *P. canis* Castellani and Chalmers, 1910. In *Canis familiaris,* Pariah dog.
11. *P. capistrani* Russell, 1932. In *Excalfactoria lineata,* island painted quail.
12. *P. caprae* (de Mello and Paes, 1923). In *Capra* sp. (*?hircus*), goat.
13. *P. carinii* *(Leger and Mouzels, 1917). In *Iguana nudicollis,* iguana.
14. *P. cathemerium* Hartman, 1927. In *Passer domesticus,* sparrow.
15. *P. caucasium* Marzinowsky, 1916. In *Homo sapiens,* man.

* A joint contribution from the Department of Biology, Peru State Teachers' College, and the Department of Zoology, Iowa State College. The study supported by a grant to the senior author from the American Association for the Advancement of Science through its affiliate the Nebraska Academy of Sciences.

16. *P. cephalophi* Bruce, Harvey, Hamerton, and Lady Bruce, 1913. In *Cephalophus grimmi,* duiker.
17. *P. cercopitheci* Theiler, 1930. In *Cercopithecus nictitans,* monkey.
18. *P. circumflexum* Kikuth, 1931. In *Turdus pilaris,* thrush.
19. *P. columbae* Carini, 1912. In *Columbia* sp., pigeon.
20. *P. cynomolgi* Mayer, 1907. In *Macacus cynomolgus,* monkey.
21. *P. diploglossi* Aragão and Neiva, 1909. In *Diploglossus fasciatus,* lizard.
22. *P. elongatum* Huff, 1930. In *Passer domesticus,* sparrow.
23. *P. epomophori* Rodhain, 1926. In *Hypsignathus monstruosus,* bat.
24. *P. equi* Castellani and Chalmers, 1913. In *Equus caballus,* horse.
25. *P. falciparum* (Welch, 1897). In *Homo sapiens,* man.
26. *P. falciparum quotidianum* Craig, 1909. In *Homo sapiens,* man.
27. *P. fallax* (Schwetz, 1930). In *Syrnium nuchale,* owl.
28. *P. giganteum* Theiler, 1930. In *Agama colonorum,* lizard.
29. *P. golgii* (Sambon, 1902). In *Homo sapiens,* man.
30. *P. gonzalezi* ♯(Iturbe, 1921). In *Anolis biporcatus,* lizard.
31. *P. grassii* Labbé, 1894. In *Alauda arvensis,* skylark.
32. *P. hexamerium* Huff, 1935. In *Sialia s. sialis,* bluebird.
33. *P. immaculatum* (Grassi and Feletti, 1890 and 1892). In *Homo sapiens,* man.
34. *P. immaculatum* Schaudinn, 1902. In *Homo sapiens,* man.
35. *P. inconstans* (Hartman, 1927). In *Passer domesticus,* sparrow.
36. *P. inui* Halberstädter and Prowazek, 1907. In *Macacus cynomolgus* and *M. nemestrinus,* monkeys.
37. *P. inui* var. *cynomolgi* Mayer, 1907 (see Sinton and Mulligan, 1933, p. 780). In *Silenus irus* (=*M. cynomolgus* of Mayer, 1907), monkey.
38. *P. inui* var. *gonderi* Sinton and Mulligan, 1932. In *Cercocebus fulginosus,* monkey.
39. *P. joyeuxi* Leger, 1928. In *Cercopithecus callitrichus,* monkey.
40. *P. knowlesi* Sinton and Mulligan, 1932. In *Silenus pileatus* (=*M. pileatus*) and *Cercopithecus pygerythrus,* monkeys.
41. *P. knowlesi* var. *sintoni* Brug, 1934. In *Macacus rhesus* and *M. cynomolgus,* monkeys.
42. *P. kochi* (Laveran, 1899). In *Cercocebus sabaeus,* monkey.
43. *P. kochi* var. *macfiei* Sinton and Mulligan, 1932. In *Papio sphinx,* monkey.
44. *P. laverani* (Labbé, 1894). In *Homo sapiens,* man.
45. *P. laverani* var. *quartana* (Labbé, 1894). In *Homo sapiens,* man.
46. *P. laverani* var. *tertiana* (Labbé, 1894). In *Homo sapiens,* man.
47. *P. mabuiae* Wenyon, 1909. In *Mabuia quinquetaeniata,* lizard.
48. *P. mackiei* de Mello and Braz de Sa, 1916. In *Vespertilio muricola,* bat.
49. *P. maculilabre* Schwetz, 1931. In *Mabuia maculilabris,* lizard.
50. *P. major* (Raffaele, 1930). In *Passer domesticus,* sparrow.
51. *P. majoris* (Laveran, 1902). In *Parus major,* great tit.
52. *P. malariae* (Laveran, 1880). In *Homo sapiens,* man.
53. P. *malariae* Marchiafava and Celli, 1885. In *Homo sapiens,* man.
54. *P. malariae immaculatum* (Grassi and Feletti, 1892). In *Homo sapiens,* man.
55. *P. malariae incolor* Schneidermühl, 1898. In *Homo sapiens,* man.
56. *P. malariae irregularis* Kruse, 1892. In *Homo sapiens,* man.
57. *P. malariae* var. *parva* (Laveran, 1901). In *Homo sapiens,* man.
58. *P. malariae* var. *quartanae* Celli and San Felice, 1891. In *Homo sapiens,* man.
59. *P. malariae* var. *quartanae* (Laveran, 1901). In *Homo sapiens,* man.
60. *P. malariae* var. *quotidianae* Celli and San Felice, 1891. In *Homo sapiens,* man.
61. *P. malariae raupachi* Parcvanidze, 1914. In *Meleagris* sp., turkey.
62. *P. malariae* var. *tertianae* Celli and San Felice, 1891. In *Homo sapiens,* man.
63. *P. malariae* var. *tertianae* (Laveran, 1901). In *Homo sapiens,* man.
64. *P. melanipherum* (Dionisi, 1899). In *Miniopterus schreibersi,* bat.
65. *P. melanipherum* var. *monosoma* Vassal, 1907. In *Vesperugo abramus,* bat.
66. *P. mesnili* ♯(Bouet, 1909). In *Naja* sp., snake.
67. *P. minasense* Carini and Rudolphi, 1912. In *Mabuia agilis,* lizard.

68. *P. murinum* (Dionisi, 1899). In *Vespertilio murinus.*
69. *P. nucleophilum* Manwell, 1935. In *Dumatella carolinensis,* catbird.
70. *P. ovale* Stephens, 1922. In *Homo sapiens,* man.
71. *P. passeris* (Johnston and Cleland, 1909). In *Passer domesticus,* sparrow.
72. *P. passeris* Grant, 1909. In *Passer domesticus,* sparrow.
73. *P. perniciosum* Ziemann, 1915. In *Homo sapiens,* man.
74. *P. pitheci* Halberstädter and Prowazek, 1907. In *Pithecus satyrus* (=*Pongo pygmaeus*), orang-utan.
75. *P. pitmani* Hoare, 1932. In *Mabuya striata* and *M. maculilarbris,* skinks.
76. *P. pleurodyniae* Small, 1924. In *Homo sapiens,* man.
77. *P. polare* Manwell, 1934. In *Petrochelidon l. lunifrons,* cliff swallow.
78. *P. praecox* (Grassi and Feletti, 1890). In *Homo sapiens,* man; *Passer domesticus* and *P. hispaniolensis,* sparrows.
79. *P. praecox* (Grassi and Feletti, 1892). In *Homo sapiens,* man.
80. *P. pteropi* Breinl, 1912? In *Pteropus gouldi,* flying fox.
81. *P. pteropi* Mackie, 1914. In *Pteropus edwardsi,* bat.
82. *P. quartana* Golgi, 1890. In *Homo sapiens,* man.
83. *P. quartanae* (Lewkowicz, 1897). In *Homo sapiens,* man.
84. *P. quotidianum* (Bates, 1913). In *Homo sapiens,* man.
85. *P. reichenowi* (Sluiter, Swellengrebel and Ihle, 1922). In a chimpanzee.
86. *P. reichenowi* Blacklock and Adler, 1924. In a chimpanzee (*?Anthropopithecus troglodytes*).
87. *P. relictum* Grassi and Feletti, 1891. In *Passer domesticus* and *P. hispaniolensis,* sparrows.
88. *P. rigolleti* Leger and Bédier, 1922. In *Myoxus murinus.*
89. *P. roubaudi* Leger and Bédier, 1923. In *Ictonyx* (=*Viverra*) *zorilla,* zorilla.
90. *P. roumei* ♯(Bouet, 1909). In *Cinixys belliana,* turtle.
91. *P. rouxi* Sergent, Ed. and Et. and Catanei, 1929. In *Passer domesticus,* sparrow and *Serinus canarius,* canary.
92. *P. sedecimanae* (Lewkowicz, 1897). In *Homo sapiens,* man.
93. *P. semnopitheci* Knowles, 1919. In *Semnopithecus entellus,* monkey.
94. *P. simondi* ♯(Castellani and Willey, 1904). In *Hemidactylus leshenaulti,* gecko.
95. *P. tenuis* (Laveran and Marullax, 1914). In *Liothrix luteus,* babbler.
96. *P. tenue* Stephens, 1914. In *Homo sapiens,* man.
97. *P. tertianae* (Danilewsky, 1895). In *Homo sapiens,* man.
98. *P. tertianae* (Lewkowicz, 1897). In *Homo sapiens,* man.
99. *P. tertianum* (Golgi, 1889). In *Homo sapiens,* man.
100. *P. tropiduri* Aragão and Neiva, 1909. In *Tropidurus torquatus,* lizard.
101. *P. tumbayaensis* (Mazza and Fiora, 1930). In *Planesticus anthracinus,* thrush.
102. *P. tyrio* de Mello, Fernandes, Correia and Lobo, 1928. In *Manis pentadactyla,* anteater.
103. *P. undecimanae* (Lewkowicz, 1897). In *Homo sapiens,* man.
104. *P. vaccinae* **Bosc, 1904. In *Homo sapiens,* man; horse, cow, *et al.*
105. *P. vassali* (Laveran, 1905). In *Sciurus grisemanus,* squirrel.
106. *P. vaughani* (Novy and MacNeal, 1904). In *Turdus migratorius,* American robin.
107. *P. vigesimo-tertianae* (Lewkowicz, 1897). In *Homo sapiens,* man.
108. *P. vivax* (Grassi and Feletti, 1890). In *Homo sapiens,* man.
109. *P. vivax* var. *minuta* Emin, 1914. In *Homo sapiens,* man.
110. *P. wasielewskii* Brumpt, 1909. In *Athene noctua,* little night owl.
111. *P. gallinaceum* Brumpt, 1935.† In *Gallus domesticus,* domestic fowl.
112. *P. paddae* Brumpt, 1935.† In *Padda oryzivora,* Java sparrow.

* Probably a Babesia (See Wenyon, 1926 p. 983).

** Found in the skin; surely not Plasmodium.

♯ Removed to genus Haemoproteus (See Wenyon, 1926, and Coatney, 1936).

† These species came to our attention after the paper had gone to press and were inserted in the galley proof.

HOST INDEX FOR THE GENUS PLASMODIUM—REPTILIA

Agama colonorum—lizard—: (2) Wenyon, 1909; (2)(28) Theiler, 1930; Schwetz, 1931.
Anolis biporcatus Bacourt—lizard—: (30) Iturbe, 1921.
Cinixys belliana—turtle—: (90) Bouet, 1909.
Diploglossus fasciatus—lizard—: (21) Aragão and Neiva, 1909.
Hemidactylus leschenaulti—gecko—: (94) Castellani and Willey, 1904.
Iguana nudicollis—iguana—: (13) Leger and Mouzels, 1917.
Iguana sapidissima—iguana—: (67) Wenyon, 1915.
Iguana tuberculata—iguana—: Scott, 1927.
Mabuia agilis—lizard—: (67) Carini and Rudolphi, 1912.
Mabuia maculilabris—lizard—: (49) Schwetz, 1931; (75) Hoare, 1932.
Mabuia Quinquetaeniata—lizard—: (47) Wenyon, 1909.
Mabuya striata—skink—: (75) Hoare, 1932.
Naja sp.—snake—: (66) Bouet, 1909.
Tropidurus torquatus—lizard—: (100) Aragão and Neiva, 1909.
Tupinambus nigropunctatus—lizard—: (13) Leger, 1919.

AVES

Acomus erythrophthalmus—: (78) Scott, 1926.
Acrocephalus steperus—: Nikitine and Artemenko, 1927.
Actophilus africanus—: Hamerton, 1930.
Agelaius phoeniceus—red-winged blackbird—: Opie, 1898.
Aidemosyne cantans—: (78) Scott, 1927.
Aidemosyne malibarica—: (78) Scott, 1926.
Alauda arvensis—sky lark—: (31) Labbé, 1894; Doré, 1922; Nikitine and Artemenko, 1927.
Alauda cristata—: Nikitine and Artemenko, 1927.
Aluco longimembris—grass owl—: (11 or 22) Russell, 1932.
Amadina erythrocephala—: (78) Scott, 1926.
Amadina fasciata—weaver finch—: Hamerton, 1934.
Anthus novae-zealandiae—lark—: Doré, 1921a.
Antigone antigone—crane—: (genus?) Scott, 1926.
Aphelocoma sordida—Swainson's blue jay—: Plimmer, 1914.
Aptenodytes patagonica—: (78) Scott, 1927.
Ara macao—red and blue macaw—: (78) Plimmer, 1912.
Aramides chiricota—: (genus?) Scott, 1926.
Argusianus argus—: (78) Scott, 1928; (78) Hamerton, 1929.
Asio leucotis—: (78) Scott, 1926.
Astur badius—: Hamerton, 1932.
Asturinula monogrammicus—: (genus?) Scott, 1927.
Athene noctua—owl—: Sergent, Ed. and Et., 1906; (110) Brumpt, 1909.
Atticora cyanolenca—: Iturbe and González, 1906.
Balearica regulorum—crowned crane—: (78) Plimmer, 1912; (78) Scott, 1926.
Barnardius semitorquata—: (genus?) Scott, 1927.
Biziura lobata—musk-duck—: (3) Gilruth, Sweet and Dood, 1910.
Bubo poensis—: (78) Scott, 1927.
Calliste thoracica—yellow-breasted tanager—: (78) Plimmer, 1912.
Calospiza fastuosa—superb tanager—: (78) Scott, 1926; Hamerton, 1929.
Caprimulgus europaeus—night jar—: (78) Böing, 1925.
Cardinalis cardinalis—: Schaudinn in Prowazek, 1911; (78) Scott, 1927; Hamerton, 1932, 1933.
Carduelis carduelis—goldfinch—: Koch, 1899.
Carduelis elegans—goldfinch—: Plimmer, 1914; Nikitine and Artemenko, 1927.
Carpodacus mexicanus—Mexican finch—: (78) Plimmer, 1912.
Carpophaga concinna—blue-tailed fruit pigeon—: (78) Plimmer, 1912; (78) Scott, 1926.
Cephalophoneus nasutus—large-nosed shrike—: Hegner and Chu, 1930.

Cerchneis tinnunculus—: Schaudinn in Prowazek, 1911.
Chamaepelia minuta—: (genus?) Scott, 1926.
Chenopsis atrata—black swan—: (3?) Cleland, 1915.
Chloris chloris—: (87) Franchini, 1924.
Chloropsis aurifrons—: (78) Scott, 1926; Hamerton, 1932.
Coccothraustes coccothraustes—hawfinch—: (87) Franchini, 1924; (78) Böing, 1925.
Coccothraustes melanura—Japanese hawfinch—: (78) Plimmer, 1912.
Coccothraustes vulgaris—: Nikitine and Artemenko, 1927.
Colaptes campestris (Vieill.)—: Carini and Maciel, 1916.
Columba palumbus—wood pigeon—: (78) Böing, 1925.
Columbia grisea—: (78) Scott, 1927.
Columbia livia—pigeon—: Sergent, Ed. and Et., 1904.
Columbia sp.?—pigeon—: (19) Carini, 1912.
Corvus b. brachyrhynchos—crow—: (14 exp.) Manwell and Herman, 1935.
Corvus corax—raven—: (78) Plimmer, 1913.
Corvus frugilegus—: Nikitine and Artemenko, 1927.
Coryllis pusillus—: Hamerton, 1930.
Crateropus bicolor—: (78) Scott, 1927.
Cryptorhinus afra—: (genus?) Scott, 1926.
Cursorius temmincki—: (genus?) Scott, 1927.
Cyanops flavifrons—yellow-fronted barbel—: Plimmer, 1914.
Cyanospiza leclancheri—rainbow bunting—: (78) Plimmer, 1912.
Cygnus melanocoryphus—swan—: Schaudinn in Prowazek, 1911; (78) Scott, 1928.
Dacnis cayana—blue sugar bird—: (78) Plimmer, 1912.
Dumatella carolinensis—catbird—: (32) Huff, 1935; (106) Manwell, 1935, (69) 1935a; (69) Manwell and Herman, 1935.
Dumetia hyperythra—: Hamerton, 1930.
Emberiza citrinella—yellow hammer—: Wasielewski, 1908.
Emberiza fucata—bunting—: (78) Plimmer, 1913.
Emberiza projer—: Wasielewski, 1902.
Emberiza variabilis—bunting—: Ogawa, 1912.
Enneoctonus collurio—: See *Lanius collurio*.
Erithragra musica—: (78) Scott, 1926.
Estrelda melpoda—orange-checked waxbill—: (78) Plimmer, 1912.
Estrilda caerulescens—: Hamerton, 1931.
Estrilda cinerea—: (78) Scott, 1926.
Estrilda phoenicotis—waxbill—: Marullaz, 1912; (78) Scott, 1926.
Euethia candra—: (78) Scott, 1926.
Euethia canora—: Hamerton, 1929.
Eulabes religiosa—starling—: (78) Scott, 1926; Hamerton, 1934.
Eunetta falcata—falcated duck—: (78) Plimmer, 1915.
Euplocanus erythrophthalmus—: Hamerton, 1929.
Excalfactoria lineata—island painted quail—: (11) Russell, 1932, 1932a.
Falco hypolencus Gould—grey falcon—: Breinl, 1912.
Francolinus bicalcaratus—: (78) Scott, 1928.
Fringilla carduelis—goldfinch—: Sergent, Ed. and Et., 1904.
Fringilla chloris—: Wasielewski, 1902.
Fringilla coelebs—chaffinch—: Wasielewski, 1902; Marullaz, 1912.
Fringilla kawarahiwa minor—: (78) Katahira, 1929.
Fringilla linota—linnet—: Sergent, Ed. and Et., 1904.
Gallus domesticus—domestic fowl—: (111) Brumpt, 1935.
Gallus gallus—common fowl—: (14, 18, 78 exp.) Manwell, 1933.
Gallus lafayettei—: Hamerton, 1932.
Gallus varius—: (genus?) Scott, 1926.
Garrulax leucolophus—jay thrush—: (78) Plimmer, 1912.
Garrulus glandarius—common jay—: (78) Böing, 1925.
Gennaeus edwardsi—pheasant—: Hamerton, 1930.

Geothlypis trichas brachidactyla—Maryland yellow throat—: (32) Huff, 1935.
Gracula religiosa—small hill mynah—: (78) Plimmer, 1912.
Haliastur indus—: Hamerton, 1932.
Hirundo rustica—: Nikitine and Artemenko, 1927.
Hylocichla musica—song-thrush—: Coles, 1914.
Hyphantorius cucullatus—weaver finch—: (78) Scott, 1926: Hamerton, 1930.
Icterus jamaicai—Brazilian hangnest—: (78) Plimmer, 1912.
Icterus tibialis—: Hamerton, 1934.
Iole gularis—Philippine bulbul—: Hegner and Chu, 1930.
Junco h. hyemalis—slate-colored junco—: (18) (78) (106 exp.) Manwell and Herman, 1935.
Lagonosticta senegala (Linné)—weaver finch—: (87) Marullaz, 1912; Hamerton, 1929.
Lamprocolius australis—: (78) Scott, 1926.
Lamprotornis aenus (= *L. caudatus*)—long-tailed glossy starling—: (78) Plimmer, 1912.
(*Lanius collurio*)—*Enneoctonus collurio*—red-backed shrike—: (78) Böing, 1925.
Lanius excubitor—great grey shrike—: (78) Böing, 1925.
Ligurinus chloris—green finch—: Ziemann, 1898.
Ligurinus crumeniferus—: (78) Scott, 1928.
Liothrix luteus—: (95) Laveran and Marullaz, 1914; (78) Scott, 1926; Hamerton, 1931.
Lissotes maculipennis—bustard—: Ross, 1911.
Loriculus indicus—: (genus?) Scott, 1926.
Loxia curvirostra—crossbill—: (78) Plimmer, 1913.
Luscinia sp.—nightingale—: Sergent, Ed. and Et., 1904.
Lyurus tetrix—blackgame—: (78) Böing, 1925.
Malimbus nitens—: (genus?) Scott, 1926.
Megacephalon maleo—: Hamerton, 1931.
Megaloprepia magnifica—: (78) Scott, 1927.
Meleagris sp.—turkey—: (61) Parcvanidze, 1914.
Melophus melanicterus—bunting—: (78) Plimmer, 1913.
Melophus melanocephalus—: (78) Scott, 1927.
Melospiza georgiana—song sparrow—: Opie, 1898.
Melospiza m. melodia—song sparrow—: (14) (18) (22) (78) (sp.?) Manwell and Herman, 1935.
Merula boulboul—grey-winged ouzel—: (78) Plimmer, 1915.
Motacilla alba—: Nikitine and Artemenko, 1927.
Munia atricapilla (Vieill.)—: (genus?) Scott, 1927.
Munia lineata—Cabani's weaver—: (11 or 22) Russell, 1932.
Munia maja Linné—: (78) Scott, 1926; Uegaki, 1930.
Munia orizizora—sparrow—: Mayer, 1910.
Munia punctulata—: Hamerton, 1932.
Munia topela (Swinh.)—: Uegaki, 1930.
Myristicivora bicolor—: (genus?) Scott, 1926.
Neotis denhami—: (78) Scott, 1927.
Nothura maculosa—: (78) Scott ,1927.
Nucifraga caryocatactes—nutcracker—: (78) Plimmer, 1912.
Numenius variegatus—eastern whimbrel—: (11 or 22) Russell, 1932.
Numida sp.—: Ross, 1911.
Numida ptilorhyncha—guinea-fowl—: Wenyon, 1908.
Nyctomassa violaceus—: (genus?) Scott, 1926.
Ortygospiza polyzona—quail finch—: (78) Plimmer, 1915.
Oryzivora oryzivorae—: (78) Katahira, 1929.
Oryzornis oryzivora—: Uegaki, 1930.
Otomela lucionensis—grey-headed shrike—: Hegner and Chu, 1930.
Padda oryzivora—Java sparrow—: (112) Brumpt, 1935a.
Paroaria cucullata—finch—: Hamerton, 1932.

Parus major—great tit—: (51) Laveran, 1902.
Passer chloris—greenfinch—: Sergent, Ed. and Et., 1904.
Passer domesticus—English sparrow—: (78) Grassi and Feletti, 1890 and 1891; MacCallum, 1898; Opie, 1898; (71) Johnston and Cleland, 1909; (72) Grant, 1909; (71) Cleland, 1915; (14) (22) (35) Hartman, 1927; Nikitine and Artemenko, 1927; (91) Sergent, Ed. and Et., and Catanei, 1929; (22) Huff, 1930; (50) Raffaele, 1930; (106 exp.) Manwell, 1934c; (14) (78) (106) (106 exp.) Manwell and Herman, 1935.
Passer hispaniolensis—: (78) Grassi and Feletti, 1890.
Passer montanus—tree sparrow—: Wasielewski, 1902; Nikitine and Artemenko, 1927.
Passer sp.—: Sergent, Ed. and Et., 1904; Mine, 1914.
Passerina ciris—snowflake—: Hamerton, 1932.
Perdix cinerea—partridge—: Laveran and Lucet, 1905.
Perdix perdix—: (78) Böing, 1925.
Pernis apivorus—honey buzzard—: Danilewsky, 1889.
Petrochelidon l. lunifrons Say—cliff swallow—: (77) Manwell, 1934.
Petrophila cinclorhyncha—: (78) Scott, 1927.
Phasianus colchicus—pheasant—: (78) Böing, 1925.
Phasianus mongolicus—Mongolian pheasant—: Plimmer, 1914.
Phonipara canora—Cuban finch—: (78) Plimmer, 1912.
Pica pica—magpie—: Kruse (according to Minchin, 1903).
Pitta novae-guineae-pitta—: (78) Plimmer, 1917.
Planesticus anthracinus (Burm.)—thrush—: (101) Mazza and Fiora, 1930.
Plesiositagra cucullata—: Hamerton, 1933.
Plocepasser mahali—: (78) Scott, 1926.
Ploceus baja (Blyth.)—: Uegaki, 1930.
Pratincola caprata—chat—: (78) Plimmer, 1913.
Prinia extensicauda (Swinhoe)—: Ogawa and Uegakhi, 1927.
Pryzivora crassirostris—: Hamerton, 1929.
Psophia crepitans—: Hamerton, 1932.
Ptelia melba—: Hamerton, 1929.
Pternistes afer humboldti—francolin—: Hamerton, 1929.
Pternistes nudicollis—: (78) Scott, 1927.
Ptilopus coronulatus—: (genus?) Scott, 1926.
Pycnonotus jocosus—red-eared bulbul—: (78) Plimmer, 1912.
Pyrrhuloxia sinuata Bonap.—Arizona pyrrhuloxia—: (78) Scott, 1928; Hamerton, 1933.
Pyromelona franciscana—: Marullaz, 1912; Hamerton, 1930.
Quele (= *?Fondia*) *aerythrops*—fire bird—: (87) Marullaz, 1912.
Quiscalus quiscula—purple grackle—: (?78) Manwell, 1934; (14) Manwell and Herman, 1935.
Rallina eurizonoides—Philippine banded crake—: (11 or 22) Russell, 1932.
Rhamphocoelus brasiliensis—: (78) Scott, 1926.
Rhea americana Linné—: Carini and Maciel, 1916.
Rhopodytes tristis—: (78) Scott, 1927.
Rollulus roulroul—: (78) Scott, 1928.
Saxicola oenanthe—: Nikitine and Artemenko, 1927.
Saxicola salex saxilaris—: Hoare, 1932.
Scardafela squamosa—scaly dove—: (78) Plimmer, 1912.
Scolopax rusticola—: (78) Scott, 1926.
Serina mozambicus—: Hamerton, 1934.
Seriniculus molinus—: (78) Scott, 1926.
Serinus canarius—common canary—: *all experimental* (78) Taliaferro, 1925; (78) Boyd, 1925; (14) (35) Hartman, 1927; (14) Boyd, 1929, 1929a; (14) (35) (78) Manwell, 1929; (91) Sergents and Catanei, 1929; (14) Taliaferro, W. H. and L. G., 1929; (22) Huff, 1930, (14) (22) (78) Manwell, 1930; (18) Kikuth, 1931; (14) (78) (91) Sergents and Catanei, 1931; (14) (22) (78) (91) Ging-

rich, 1932; (22) Hartman, 1932; (14) (22) (78) (91) Manwell, 1932; (11) Russell, 1932; (14) Boyd, 1933; (14) (18) (22) (69) (78) (91) Manwell, 1934a; (32) Huff, 1935; (106) Manwell, 1935; (11) (77) (106) Manwell, 1935a; (14) (18) (22) (69) (77) (78) (106) Manwell and Herman, 1935.
Serinus hortulanus—: (78) Scott, 1927.
Serinus icterus—: (78) Scott, 1927.
Sialia sialis—: (78) Scott, 1926, 1927; (32) Huff, 1935.
Sitagra luteola—slender-billed weaver—: Hamerton, 1933.
Spermospiza haematina—: Hamerton, 1932.
Spinus citrinellus—siskin—: Schaudinn in Prowazek, 1911.
Spizella p. passerina—chipping sparrow—: (22) (78) (sp.?) Manwell and Herman, 1935.
Sporaeginthus amandava—weaver finch—: Hamerton, 1930
Sporaeginthus melopodes—: (78) Scott, 1926.
Spreo superbus—: (78) Scott, 1926, 1927.
Stoparola melanops—fly catcher—: (78) Plimmer, 1912.
Streptopelia semitorquatus—: (genus?) Scott, 1926.
Strix otus—long-eared owl—: Wasielewski, 1902.
Strix flammea—barn owl—: Sergent, Ed. and Et., 1906.
Sturnus vulgaris—common starling—: (?78) Manwell, 1934; (14) Manwell and Herman, 1935.
Sturnus vulgaris menzbieri—: (78) Scott, 1926.
Suthora gularis—: Hamerton, 1931.
Sylvia atricapilla—blackcap warbler—: Sergent, Ed. and Et., 1904.
Synallaxis ruficapilla (Vieill.)—: Carini and Maciel, 1916.
Syrmaticus reevesi—: (78) Scott, 1927.
Syrinum aluco—tawny owl:—Sergent, Ed. and Et., 1906.
Syrnium nuchale—owl—: (27) Schwetz, 1930, 1931.
Textor alector—ox-bird—; (78) Plimmer, 1912.
Thrush—: Coles, 1914.
Totanus eurhinus—Asiatic redshank—: (11 or 22) Russell, 1932.
Toxostoma cinereum—thrasher—: (78) Plimmer, 1913.
Tragopan satyra—: (78) Scott, 1926.
Trochalopterum taiwanum (Swinehoe)—:Ogawa and Uegaki, 1927.
Turacus corythaix—white crested touracou—: Plimmer, 1914.
Turdus merula—blackbird—: Doré, 1921, 1922; (18) (95) Giovannola, 1934.
Turdus migratorius—American robin—: (106) Novy and MacNeal, 1905; (78) Plimmer, 1916; (106) Manwell, 1934c, 1935; (18) (78) (106) Manwell and Herman, 1935.
Turdus musicus—song thrush—: Doré, 1922.
Turdus mustelinus—wood thrush—: Plimmer, 1914.
Turdus pilaris—thrush—: (18) Kikuth, 1931.
Turkey (sp.?)—: (61) Parcvanidze, 1914.
Turnix fasciata—Philippine button quail—: (22) Russell, 1932.
Turtur auritus—dove—: Sergent, Ed. and Et., 1904.
Turtur orientalis—dove—: Ogawa, 1912.
Tympanistria bicolor—: tambourine pigeon—: (78) Plimmer, 1912.
Tympanistria tympanistria—dove—: (78) Scott, 1927; Hamerton, 1932.
Uraeginthus bengalus—weaver finch—: Hamerton, 1934.
Zenaidura macroura macroura—mourning dove—: (32) Huff, 1935.
Zonotrichia albicollis—white-throated sparrow—: (14) (18) Manwell and Herman, 1935.
Zonotrichia leucophrys gambelli—: (78) Scott, 1926.
Zosterops palpebrosa peguensis Baker—white-eye—: Ogawa and Uegaki, 1927.

Mammals

Achromaticus vesperuginis—bat—: (1) Yakimoff and Stolïnioff, 1912.
Alouatta palliata inconsonans Goldman—Panama howling monkey—: (*P.?*) Clark,

1931, (5) 1934 (see Taliaferro and Taliaferro, 1934); (5) Taliaferro and Taliaferro, 1934.
Alouatta p. palliata—monkey—: (5) Clark, 1934 (see Taliaferro and Taliaferro, 1934).
Anthropopithecus gorilla—gorilla—: (25) (108) Reichenow, 1920.
Anthropopithecus troglodytes—chimpanzee—: (42) Lühe, 1906; (25) (52) (108) Reichenow, 1920; (25?) Blacklock and Adler, 1922; (86) Ibid., 1924; (25?) Adler, 1923.
Aotus zonalis—night monkey—: (5 exp.) Taliaferro and Taliaferro, 1934.
Ateles sp.—: Seidelin, 1912.
Ateles dariensis Goldman—black spider monkey—: (species? exp.) Clark, 1931; (5) Clark, 1934 (see Taliaferro and Taliaferro, 1934).
Ateles geoffroyi Kuhl—red spider monkey—: (108-like) Clark, 1930; (species?) Clark, 1931; (5) Taliaferro and Taliaferro, 1934.
Brachycerus calvus—: (6) Gonder and von Gossler, 1909.
Buffalo, plains—(?Bubalis sp.)—: (8) Sheather, 1919; (8) Edwards, 1925 (see Wenyon, 1926).
Cacajoa (= *Brachyurus*) *calvus*—monkey—: (5) Gonder and von Berenberg-Gossler, 1908.
Callithrix personata—monkey—: (42) (42 exp.) Anderson and Cowdry, 1928; (42) (42 exp.) Cowdry and Cowell, 1928.
Callosciurus prevosti—: (105) Scott, 1927; Hamerton, 1930, 1931.
Canis familiaris—dog—: (10) Castellani and Chalmers, 1910.
Capra (*?hircus*)—goat—: (12) de Mello and Paes, 1923.
Cebus capucinus—white-throated capuchin—: (5) Taliaferro and Taliaferro, 1934.
Cebus capucinus capucinus (Linné)—Columbian white-throated capuchin—: (species?) Clark, 1931.
Cebus capucinus imitator Thomas—Panama white-throated monkey—: (52-like) Clark, 1930; (species?) Clark, 1931.
Cephalophus grimmi—antelope—: (16) Bruce, *et al.*, 1913, 1915.
Cercocebus aetheopicus—mangabey—: (42) Plimmer, 1916; (42) Grigorieva, 1929.
Cercocebus agilis—monkey—: (42?) Schwetz, 1933a.
Cercocebus atterrimus—monkey—: (42?) Schwetz, 1933a.
Cercocebus fuliginosus—: (42) (42 exp.) Gonder and von Gossler, 1908; (42) (42 exp.) Berenberg-Gossler, 1909; (42 exp.) Gonder and Rodenwaldt, 1910; (38) Sinton and Mulligan, 1932.
Cercocebus palliatus cottoni—monkey—: (42?) Schwetz, 1933a.
Cercocebus sabaeus—: (42) Laveran, 1899.
Cercocebus sp.—monkey—: (42) Koch, 1906 (see Wenyon, 1926); (42?) Schwetz, 1933a.
Cercopithecus albogularis—: (42) Sergent, 1908.
Cercopithecus callitrichus—: (36 exp.) Leger and Bouilliez, 1912, 1913; (36 exp.) Bouilliez, 1913; (42?) Leger and Bouilliez, 1913; (42) Joyeux, 1913; (42) Bouilliez, 1916; (39) Leger, 1928.
Cercopithecus campbelli—: (4) Leger, 1922.
Cercopithecus cephus—: (36 exp.) Leger and Bouilliez, 1912, 1913; (36 exp.) Bouilliez, 1913; (42) Ringenbach, 1914.
Cercopithecus diana—monkey—: Strong and Shattuck, 1930; (42) Theiler, 1930.
Cercopithecus erythrotis—: (42) Scott, 1926.
Cercopithecus kandti—monkey—: (42?) Schwetz, 1933a.
Cercopithecus leucampyx—monkey—: (42?) Schwetz, 1933a.
Cercopithecus mona—: Seidelin and Connal, 1914; Connal and Coghill, 1916.
Cercopithecus nictitans—monkey—: Strong and Shattuck, 1930; (17) Theiler, 1930.
Cercopithecus pygerythrus—: (42) Kinghorn and Yorke, 1912; (42) Wenyon, 1926; (species? exp.) Knowles and Das Gupta, 1932; (42) (42 exp.) Napier and Campbell, 1932; (40) Sinton and Mulligan, 1932.
Cercopithecus abaeus—green monkey—: Koch, 1898; Kossel, 1899; Ziemann, 1900; (42) Berenberg-Gossler, 1909; (42) Martoglio, *et al.*, 1910; (42) Plimmer, 1912.

Cercopithecus schmidti—monkey—: (42?) Schwetz, 1933a.
Cercopithecus, sp.—: (42) Kossel, 1899; (42) Bruce and Nabarro, 1903; (42) Dutton, *et al.,* 1906; (42) Koch, 1906 (see Wenyon, 1926); (42) Gray and Tullock, 1907; (42) Ross, 1907; (20 exp.) Mayer, 1908.
Chimpanzee sp.—: (85) Sluiter, *et al.,* 1922; (86) Blacklock and Adler, 1924; (25) (52) (108) Schwetz, 1933; (25) (95) (108) Schwetz, 1934.
Colobus rufomitratus—monkey—: Strong and Shattuck, 1930.
Cow—: (104) Bosc, 1904.
Cynocephalus anubis (=*Papio anubis*)—monkey—: (36 exp.) Leger and Bouilliez, 1912, 1913.
Cynocephalus sp.—monkey—: (see also *Papio*); Kossel, 1899; (42?) Schwetz, 1933a.
Epomophorus franqueti-franqueti Tomes—bat—: by error—Rodhain, 1915 (see *Hypsignathus monostruosus* H. Allen); (23) Rodhain, 1926a.
Epomophorus gambianus—: (80) Leger, A. and M., 1914.
Epomophorus wahlbergi haldemani Hallowell—bat—: (23) Rodhain, 1926a.
Equus caballus—: (24) Castellani and Chalmers, 1913.
Erythrocebus patus—monkey—: (36 exp.) Leger and Bouilliez, 1912, 1913; (42) (see Stiles and Nolan, 1929).
Goat (*Capra ?hircus*)—: (12) de Mello and Paes, 1923.
Homo sapiens—man—: (52) Laveran, 1880; (53) Marchiafava and Celli, 1885; (99) Golgi, 1889; (33) (78) (108) Grassi and Feletti, 1890; (60) Celli and San Felice, 1890; (82) Golgi, 1890; (58) (60) (62) Celli and San Felice, 1891; (33) (54) (79) Grassi and Feletti, 1892; (56) Kruse, 1892; (44) (45) (46) Labbé, 1894; (97) Danilewsky, 1895; (25) Welch, 1897; (83) (92) (98) (103) (107) Lewkowicz, 1897; (55) Schneidermühl, 1898; (57) (59) (63) Laveran, 1901; (29) Sambon, 1902; (29) Sambon and Lou, 1902; (34) Schaudinn, 1902; (104) Bosc, 1904; (26) Craig, 1909; (84) Bates, 1913; (96) Stephens, 1914; (109) Emin, 1914; (9) (73) Ziemann, 1915; (15) Mariznowsky, 1916; (70) Stephens, 1922; (76) Small, 1924; (sp. exp.) Knowles and Das Gupta, 1932.
Horse—: (104) Bosc, 1904.
Hylobates hoolock—gibbon—: Knowles and Das Gupta, 1932.
Hypomelanus enganensis—: (80) Scott, 1926.
Hysignathus monstruosus H. Allen—bat—: Rodhain, 1915 (see *Epomophorous franqueti-franqueti*); (23) Rodhain, 1926a.
Ictonyx (=*Viverra*) *zorilla*—zorilla—: (89) Leger and Bédier, 1923.
Leontocebus geoffroyi—marmoset—: (5 exp.) Taliaferro and Taliaferro, 1934.
Macacus—: see also *Silenus.*
Macacus cynomolgus (=*Silenus irus*)—monkey—: (36) Halberstädter and Prowazek, 1907; (20) Mayer, 1907, 1908; (20 exp.) Flu, 1908; (20 exp.) Mayer, 1908; (20 exp.) Blanchard and Langeron, 1912; (36 exp.) Leger and Bouilliez, 1912, 1913; (36 exp.) Bouilliez, 1913; Franchini, 1927; (36?) Kingsbury, 1931; Green, 1931, 1932;; (36) (40) (41) Brug, 1934; (20) (36) (40) Sinton, 1934.
Macacus (?) *cyclopis* (Swinhoe)—monkey—: Arima, 1933.
Macacus lasiotis tcheliensis—: (36) Mathis and Leger, 1911.
Macacus nemestrinus—: (36) Halberstädter and Prowazek, 1907; (36 exp.) Leger and Bouilliez, 1912, 1913; (36 exp.) Bouilliez, 1913; Green, 1932.
Macacus rhesus (=*Silenus rhesus*)—monkey—: Bruce and Nabarro, 1903; (20 exp.) Mayer, 1908; (36) Mathis and Leger, 1911, 1911a; (36 exp.) Leger and Bouilliez, 1912, 1913; Chimisso, 1922; (sp. exp.) Knowles and Das Gupta, 1932; (?42 exp.) Napier and Campbell, 1932; (?) Arima, 1933; (37 exp.) (40 exp.) Sinton and Mulligan, 1933; (36, 40, and 41 exp.) Brug, 1934.
Manis pentadactyla—: (102) de Mello, *et al.,* 1928.
Micropteropus pusillus Peters—dwarf bat.—: (23) Rodhain, 1926a.
Minopterus schreibersi—bat—: Dionisi, 1898, (64) 1899.
Monkey—: Duke, 1921.
Myotis capaccinii—: (64) Schingareff, 1906.
Myoxus murinus—: (88) Leger and Bédier, 1922.

Nycteris hispida Schreber—bat—: Rodhain, 1926a.
Papio—: see also *Cynocephalus.*
Papio anubis (=*Cynocephalus anubis*)—monkey—: (36 exp.) Leger and Bouilliez, 1912, 1913.
Papio babuinus—: Koch, 1898; Kossel, 1899.
Papio pruinosus—: Lamborn, 1929.
Papio sphinx—: Seidelin and Connal, 1914; Connal and Coghill, 1916; (42) Leger, 1928; (36?) Macfie, 1928; (36?) Grigorieva, 1929; (43) Sinton and Mulligan, 1932.
Petalia grandis—: Strong and Shattuck, 1930; Theiler, 1930.
Petrodromus tetradactylus—rat—: (7) Rodhain, *et al.,* 1913.
Petrodromus venustus—: Bruce, *et al.,* 1915.
Pithecus satyrus (=*Pongo pygmaenus*)—orang-utan—: (74) Halberstädter and Prowazek, 1907.
Pongo—: see also *Pithecus.*
Presbytes—: see also *Pygathrix* and *Semnopithecus.*
Presbytes pileatus—: (93) Scott, 1926; (93) Wenyon, 1926.
Pteropus edwardsi—: (81) Mackie, 1914.
Pteropus gouldi—flying fox—: (80) Breinl, 1912.
Pteropus medius—: (80) Scott, 1927.
Pteropus natolis—: Durham, 1908.
Pygathrix—: see also *Presbytes* and *Semnopithecus.*
Pygathrix schistaceus—monkey—: (40 exp.) Sinton and Mulligan, 1933, 1933a.
Sciurus grisemanus—: (105) Vassal, 1905; (105) Laveran, 1905.
Sciurus vittatus—: (105) Vassal, 1907a.
Sciurus sp.—: (105) Vassal, 1907a.
Semnopithecus—: see also *Presbytes* and *Pygathrix.*
Semnopithecus entellus—monkey—: (93) Knowles, 1919; (species exp.) Knowles and Das Gupta, 1932.
Silenus—: see also *Macacus.*
Silenus irus—monkey—: see also *Macacus cynomolgus;* (36?) Noguchi, 1928; (42?) according to Stiles and Nolan, 1929; (36?) Green, 1932; (sp.) (sp. exp.) Knowles and Das Gupta, 1932; (42?) Napier and Campbell, 1932; (37 exp.) (40) Sinton and Mulligan, 1933; (20?) Sinton and Mulligan, 1932a; (37) (40) Mulligan and Sinton, 1933; (20) (40) Ibid., 1933a; (37) (40) (37 and 40 exp.) Sinton and Mulligan, 1933a; (20) (40) Sinton and Harbhagwan, 1935; (20) (36) (40 exp.) Sinton, 1935.
Silenus irus mordax—: (36?) Green, 1932.
Silenus pileatus—: (93) according to Stiles and Nolan, 1929; (40) Sinton and Mulligan, 1932.
Silenus rhesus—: see also *Macacus rhesus;* (20 exp.) Sinton and Mulligan, 1932a; (37 and 40 exp.) Mulligan and Sinton, 1933; (40 exp.) Ibid., 1933a; (37 and 40 exp.) Sinton and Mulligan, 1933a; (20 and 40 exp.) Sinton and Harbhagwan, 1935; (40) Sinton, 1935.
Silenus sinicus—: Donovan, 1920; (36 exp.) Leger and Bouilliez, 1912, 1913; (sp. exp.) Knowles and Das Gupta, 1932; (37 and 40 exp.) Mulligan and Sinton, 1933; (40 exp.) Ibid., 1933a; (40 exp.) Sinton and Mulligan, 1933; (37 and 40 exp.) Ibid., 1933a; (20 and 40 exp.) Sinton and Harbhagwan, 1935.
Silenus sp.—: (36 exp.) Halberstädter and Prowazek, 1907; (36 exp.) Mathis and Leger, 1911.
?Silenus sp.—: (42) Castellani and Chalmers, 1913.
Simia satyra—: (74) Scott, 1926.
Ursus sp.—bear—: Yakimoff, *et al.,* 1910.
Vespertilio capensis—: (68) Bowhill, 1906.
Vespertilio daubentoni—: (68) Schingareff, 1906.
Vespertilio muricola—bat—: (48) de Mello and Braz de Sa, 1916.
Vespertilio murinus—: Dionisi, 1898, (68) 1899.
Vesperugo abramus—bat—: (65) Vassal, 1907.
Viverra—: see also *Ictonyx.*

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THE MORPHOLOGY AND LIFE HISTORY OF *PLAGITURA PARVA* STUNKARD, 1933, (TREMATODA)*

HORACE W. STUNKARD

INTRODUCTION

In preliminary papers, Stunkard (1933a, 1933b) reported on the morphology and life history of a new species, *Plagitura parva,* which occurs in the intestine of the vermillion spotted newt, *Triturus viridescens.* The conclusions presented in the preliminary reports are now supported by more complete data.

The genus *Plagitura* was erected by Holl (1928) to contain a species found in *T. viridescens* near Durham, North Carolina. The parasite, which he named *P. salamandra,* was reported from both the aquatic and terrestrial stages of the salamander. The description of the species is based on the morphology of the type specimen which is represented in his Figure 1.

Other specimens were reported by Holl which differ considerably from the type specimen and it is doubtful whether they all belong to the same species. The specimen from the left, shown in Holl's Figure 2 is much larger than the type specimen. The gonads, however, are small and the uterus is apparently empty. The specimen shown in his Figure 3 is probably a young individual as the excretory system can be observed more readily before the body becomes filled with reproductive structures. These specimens, I believe, belong to the species, *P. salamandra.* In my opinion, the small gravid worms, reported by Holl and shown in his Figure 4, can not be assigned to the same species as the large ones and these small individuals appear to be identical with those I have described (Stunkard 1933a) as *Plagitura parva.*

Hundreds of specimens of *Plagitura* have been collected from newts taken near New York. This material contains many individuals which can be definitely determined as *P. salamandra,* but more than half of the worms belong to a differentiable group that manifests striking uniformity in size and internal relations. They are distinctly smaller than *P. salamandra,* the suckers are smaller, the body musculature is more delicate, while the gonads are relatively larger and situated more posteriorly in the body. These differences can not be explained by development in another host species or by differences in degree of maturity. They are regarded as distinguishing characters of a second species for which the name *P. parva* was proposed (Stunkard 1933a).

Opportunity has been afforded to compare specimens collected in

* Contribution from the Biological Laboratory, New York University.

the New York area with material from other regions. Dr. Holl kindly sent specimens of *Plagitura* collected in North Carolina. The material contains representatives of the large form which he designated as *P. salamandra* and the small sexually mature form shown in his Figure 4. Through the kind interest of Mr. Thomas D. Brown of the University of Virginia, I received twelve mounted specimens of *Plagitura* and ten living newts collected near Charlottesville, Virginia. Nine of the newts were infected and provided additional material. The specimens received from Mr. Brown may also be separated into two groups, one of which is identical with *P. salamandra* and the other with *P. parva.* In the latter group there is one sexually mature specimen which measures only 0.78 mm. in length.

In a preliminary paper, Kelley (1934) reported the collection of *P. salamandra* from newts in Massachusetts, Pennsylvania, Ohio and Virginia. Her account was concerned chiefly with the great variation found in these specimens. The author noted significant differences in size of body and size of suckers, together with variations in size, form and location of the gonads. She pointed out that average measurements made on her specimens are smaller than corresponding ones given by Holl. Kelley studied the specimens of *P. salamandra* deposited by Holl in the U. S. National Museum and contrary to his statement that the cuticula is unarmed, found the anterior portion of the body covered with minute spines.

All trematode species manifest slight variations but the differences which occur in *Plagitura* material do not have the earmarks of intraspecific variations. An examination of Holl's figures leads to doubt concerning the specific identity of his material. The difficulties encountered by Kelley may be explained and removed by the recognition of two species in the genus *Plagitura.* The species *P. salamandra* and *P. parva* can not be distinguished on size differences alone for the limits of the two species overlap. Large specimens of *P. parva* may be larger than small specimens of *P. salamandra,* but an examination of the suckers and reproductive organs will show the specific features. For example, if two worms of the same size but of different species are compared, the specimen of *P. parva* will have smaller suckers and show greater development of the gonads. The morphological differences between *P. salamandra* and *P. parva* are portrayed in the camera lucida drawings, (Figs. 1 and 2) made at the same magnification, of representative examples of the two species.

A possible bionomic distinction between the two species may be mentioned. The life cycle of *P. parva* has been completed in the laboratory and the larval stages are described in the present paper. Similar attempts to trace the life cycle of *P. salamandra* have yielded only negative results.

The bionomic distinction is made with reservation, however, since more failures than successes have resulted from the life history experiments with *P. parva.* Under laboratory conditions, which are much less constant and uniform than those of nature, with relatively small amounts of water and correspondingly more rapid changes in temperature, hydrogen ion concentration, accumulation of excretory and other wastes, etc., it is often exceedingly difficult to complete a life history even when all the stages are known.

Plagitura salamandra Holl, 1928

Examination of the original material of *P. salamandra* sent by Dr. Holl and of many additional specimens collected in Virginia, Pennsylvania, New Jersey, and New York permits certain additions to and emendations of the specific description. One of the specimens received from Dr. Holl is shown (Fig. 1). It measures slightly over 3 mm. in length and is obviously somewhat flattened. The smallest gravid specimen measures 1.5 mm. in length and 0.4 mm. in width. It was apparently just reaching sexual maturity as the gonads are very small and there are only a few eggs in the uterus. Sexually mature specimens of *P. salamandra* measure from 1.5 to 4 mm. in length. The average size of the organs agrees with the measurements given by Holl. Confirming the report of Kelley, spines were found arranged in regularly alternating rows on the anterior portion of the body. The size of eggs given by Holl is 0.035 by 0.012 mm. The eggs are operculate and vary from 0.03–0.035 mm. in length and from 0.017–0.022 mm. in width.

Plagitura parva Stunkard, 1933

Diagnosis: *Plagitura,* sexually mature specimens 0.78–2.3 mm. long. Gonads relatively larger and farther posteriad than in *P. salamandra.*

Hosts: Sexually mature stage in intestine of *Triturus viridescens;* cercaria develop in sporocysts in *Helisoma antrosa;* metacercaria are found encysted in *Physella heterostropha, Pseudosuccinea columella, Lymnaea stagnalis, Campeloma sp.,* and insect larvae.

Locality: New York, New Jersey, Pennsylvania, Virginia and North Carolina.

Type Specimen: In Helminthological Collection, Department of Lower Invertebrates, American Museum of Natural History, New York. Cotypes in author's collection.

LIFE HISTORY STUDIES

The salamander, *Triturus viridescens* Rafinesque, is common in ponds near New York. These animals harbor three species of adult

trematodes, a species of *Diplodiscus* in the cloaca, and the two species of *Plagitura* in the intestine. Eggs of the flukes may be found in large numbers on the bottom of small aquaria in which newts are kept. The eggs of *Diplodiscus* are large and hatch soon after they are passed; those of *Plagitura* are small and do not hatch in water.

My colleague, Dr. Chas. H. Willey has been studying the morphology and life history of an amphistome cercaria found in *Helisoma antrosa* collected from a pond in the Pocono Mountains, Pennsylvania, and which he suspected of being a larval stage of *Diplodiscus*. In the course of his study he placed newts, taken from the same pond, which were passing eggs of both *Diplodiscus* and *Plagitura,* in an aquarium with laboratory raised, uninfected specimens of *Helisoma antrosa.* About six weeks later, cercariae began to emerge from the snails but they were not larvae of *Diplodiscus* and it appeared probable that they were cercariae of one of the species of *Plagitura.*

Meanwhile, I had been studying the larval trematodes which infest the snails of a pond near Cold Spring on the Hudson, New York, and in *H. antrosa* had found a species that was very similar to the one which appeared in Dr. Willey's aquarium. Further study has confirmed their identity and disclosed the life history of the parasite. Newts are abundant in the pond near Cold Spring and almost every one examined contained specimens of *Plagitura.* Other newts were collected from the pond in the Pocono Mountains and specimens of *Plagitura* were found in twenty-four out of twenty-seven animals.

To test the hypothesis that the cercaria collected near Cold Spring is a larval stage of one of the species of *Plagitura,* newts from that pond were dissected and gravid worms of both species were collected and placed in separate dishes. In a few hours large numbers of eggs were shed and they could not be distinguished from those previously collected from the aquaria in which the newts were kept. The shells are thick, dark colored, and too opaque for satisfactory study of the miracidia. When eggs are passed from the newt, the miracidia are usually mature although two or three days at room temperature may be required before the larvae become active. Such eggs do not hatch in tap water although they have been kept under observation for long periods of time. Attempts to hatch the miracidia in various solutions have been unsuccessful. The larvae have been forced out by pressure on the eggs, but such miracidia were abnormal in appearance and soon disintegrated.

Eggs of *Plagitura parva* containing moving miracidia were placed in a dish with five laboratory raised specimens of *Helisoma antrosa* on November 23rd, 1932, and cercariae began to emerge from the snails on December 20th. Three became infected. The experiment was repeated on December 5th using ten snails and cercariae began to emerge on

January 6th. Five snails became infested. On February 28th, 1933, several hundred eggs of *P. parva* were placed in a small aquarium with five laboratory raised specimens of *H. antrosa.* One snail was killed and fixed on March 2nd. This snail was cut in serial sections and twenty-four eggs were found in the intestine. In eighteen of these eggs the operculum was open and the miracidium had emerged. Some of the miracidia were found in the lumen of the intestine and one was penetrating the intestinal wall. The other snails were killed 6, 10, 15, and 20 days after exposure. All were cut in serial sections but no infection was found. Although these experiments failed to provide the early stages in the snail, they demonstrated that *Helisoma antrosa* is the natural host and that the snails become infected by eating the eggs of the fluke. The experimentally infected snails continued to live for several months and provided cercariae for other experiments.

The cercariae do not encyst in the water and die in about 24 hours. Since the larva is provided with a stylet, penetration and cystogenous glands, it appeared probable that some animal which serves as food for the newt serves also as the second intermediate host of the parasite. Accordingly, on October 25th, 1932, normally emerged cercariae from a natural infection were placed in small aquaria with dragon fly, caddis fly and dobson fly larvae. The dragon fly and caddis fly larvae did not become infected but a large number of encysted metacercariae were recovered from the body cavity of the dobson fly larvae. These cysts were fed to a newt that had been kept for several weeks in the laboratory. The animal was killed three days later and the excysted metacercariae were found in the intestine.

Newts regularly feed on snails as well as insect larvae. One of the specimens of *H. antrosa,* which had been experimentally infected and from which cercariae were escaping, was placed on January 6th, 1933, in an aquarium with laboratory raised *Physella heterostropha.* Specimens of *P. heterostropha* removed two days later contained from 6 to 14 encysted metacercariae in the foot and mantle. Specimens removed a month later contained as many as 50 metacercariae. Experimentally infected specimens of *H. antrosa* were placed also in small aquaria with specimens of *Lymnaea stagnalis, Pseudosuccinea columella,* and *Campeloma sp.* The cercariae penetrated and encysted in the foot and mantle of all these snails. At the end of three weeks there were about a dozen metacercariae in each of the *L. stagnalis* and *P. columella,* and about 6 in each of the specimens of *Campeloma sp.* In no instance were metacercariae observed in the foot or mantle of *Helisoma antrosa.*

Attempts to infect frog tadpoles and young newts were unsuccessful as the cercariae did not penetrate into these animals.

To determine how soon after encystment the metacercariae are infec-

tive for newts, a laboratory raised, uninfected specimen of *P. heterostropha* was placed in a dish with recently emerged cercariae on February 13th, 1933, and fed to a newt on February 15th. The newt was killed two days later and six very small worms were recovered from the intestine. They were alive and active and it is apparent that the metacercariae are infective very soon after encystment.

Reference is made earlier to a possible bionomic difference between the two species of *Plagitura*. In the life history experiments, eggs were collected from gravid specimens of both *P. parva* and *P. salamandra*. It is probable that some of the eggs shed by the worms contained immature miracidia and that such larvae are not infective. The *P. parva* eggs, however, yielded successful results as reported. The same methods were employed with the eggs of both species, but the results with the *P. salamandra* eggs were entirely negative.

Eggs of *P. salamandra* were placed in spring water for three days and then transferred to small aquaria with laboratory raised specimens of *H. antrosa*. Various other species of snails have been used in similar experiments. All attempts to infect snails with eggs of *P. salamandra* have so far proved unsuccessful. It is probable that a species of *Helisoma* is the natural host of this parasite and the problem is still under investigation.

A stylet cercaria, similar in appearance but slightly larger than the cercaria of *P. parva,* has been found repeatedly in *H. antrosa* collected from ponds in which there were newts that harbored *P. salamandra*. At first it appeared probable that this cercaria is the larval stage of *P. salamandra*. These cercariae penetrated into and encysted in various species of snails but the metacercariae did not excyst when fed to newts. The metacercariae, still within their cysts, were recovered in large numbers after they had passed through the digestive tract of the newts.

THE LIFE CYCLE OF PLAGITURA PARVA

Morphology of the sexually mature stage

Specimens of *P. parva* are flattened dorsoventrally, elongate oval in outline. The shape varies with the extension and retraction of different parts of the body. When removed from the intestine of their host, the worms are very active and live for two or three days in isotonic salt solutions. Fixed and mounted gravid specimens (Fig. 2) measure from 0.8–2.3 mm. in length and 0.4–0.6 mm. in width. The preacetabular portion of the body is covered with spines arranged in regularly alternating rows. They are largest near the anterior end and diminish in size posteriorly. The acetabulum is situated at the caudal end of the anterior third of the body and measures from 0.15–0.2 mm. in diameter.

Digestive System.—The oral sucker is subterminal, 0.12–0.15 mm.

in diameter. There is a short prepharynx and the pharynx measures from 0.08–0.1 mm. in diameter. The esophagus is long, the bifurcation of the digestive tract is lambdoid and situated about midway between the acetabulum and the pharynx. The pharynx and anterior part of the esophagus are surrounded by clusters of deeply staining cells. The ceca terminate just in front of the testes and are relatively longer than those of *P. salamandra.*

Excretory System.—In adult specimens the body is so filled with reproductive structures and secretory cells that the study of this system is almost impossible. In the cercaria the tissues are so delicate that they break down before a sufficient amount of pressure can be exerted to demonstrate the finer tubules. In excysted metacercariae and in young worms, however, it has been possible to work out the details of this system.

The excretory system (Fig. 11) manifests the typical plagiorchid pattern. The pore is terminal and the vesicle is Y-shaped, dorsal in position. Its wall is thin and flexible and the vesicle readily changes shape with the accumulation of fluid and the pressure of surrounding structures. In young specimens particularly, the stem of the organ often shows many large lateral outpocketings. The vesicle bifurcates a short distance behind the ootype and the crura, although short, may extend forward on either side to the level of the acetabulum. From each of the crura, at the level of the ootype, a common collecting duct passes laterally, ventral to the cecum. The duct is long and much coiled; lateral to the cecum it divides into anterior and posterior branches. The proximal portion of the duct is ciliated and the beat of the cilia is toward the vesicle. In young specimens the proximal portions of the anterior and posterior collecting ducts are enlarged.

The arrangement of the excretory system is similar in each of the four quadrants of the body, (Fig. 11). On either side, the anterior collecting duct passes cephalad to the level of the bifurcation of the digestive tract where it divides into a recurrent branch and one which continues forward. The recurrent branch gives off a tubule and then divides to form two additional tubules. These three tubules and the flame cells in which they terminate supply the preacetabular area. The duct from which the recurrent branch arose continues forward for a short distance and then divides. One of the resultant limbs is subdivided into three tubules in the same manner as that described for the recurrent branch. The three cells at the distal ends of these tubules drain the esophageal area. The other limb of the anterior branch passes forward and is subdivided in a similar manner to supply three flame cells which lie in the region of the pharynx and oral sucker.

On either side the posterior collecting duct and its branches manifest

an arrangement which is an exact counterpart of the corresponding portion of the excretory system in the anterior half of the worm. There are three groups, each consisting of three flame cells, in each quadrant of the body. The flame cell formula of *Plagitura parva* is $2 \times (3+3+3) + (3+3+3)$; the worm contains thirty-six of these cells and their arrangement is shown in Fig. 11.

Male Reproductive System.—The testes are spherical to oval, with the surface indented and sometimes lobed. They are situated on opposite sides of the body, immediately behind the ends of the intestinal ceca. The shape is not rigid, and they may elongate in either axis as the worm extends and contracts. They vary from 0.16–0.26 mm. in diameter. Sperm ducts pass forward from the anterior margins and unite as they enter the cirrus sac. Within the sac the common sperm duct widens to form a coiled seminal vesicle (Fig. 2), and contracts to a narrow tube at the region of the cirrus. The cirrus is probably eversible although it was not observed in that condition. The common genital pore is situated just behind the bifurcation of the digestive tract, slightly left of the median plane. The opening of the metraterm is in front and at the left of the opening of the cirrus sac. The cirrus sac may extend almost to the ovary, although usually its caudal end is dorsal to the acetabulum.

Female Reproductive System.—The ovary is spherical to oval; its contour is notched but not lobed. It is situated slightly on the right side of the body and partially overlies the caudal margin of the acetabulum. As a result it is frequently diagonal in position with the anterior end pushed laterad and the posterior end more mediad. It measures from 0.12–0.2 mm. in length and from 0.11–0.16 mm. in width. The oviduct arises at the dorsal, median, posterior face of the ovary and passes mediad and ventrad. Its central portion is expanded to form a fertilization space and distally it communicates with the ootype. On the right side there is a diverticulum, the seminal receptacle, which is about one-third to one-half the size of the ovary and partially overlies that organ. Near the communication with the seminal receptacle, Laurer's canal is given off. The proximal portion of the canal is enlarged and frequently contains spermatozoa. Laurer's canal passes ventrad, caudad, and then turns dorsad, opening to the surface near the median line at the level of the caudal margin of the ovary. After the origin of Laurer's canal the female duct passes ventrally where it receives a short duct from the vitelline receptacle and then it turns caudad to become the initial portion of the uterus. The ootype is enclosed in the cells of Mehlis' gland. The vitellaria consist of uninterrupted masses of follicles which occupy the lateral areas of the body from the level of the intestinal bifurcation to that of the testes. On either side there are anterior and

posterior collecting ducts (Fig. 2) which converge as they pass mediad. They unite at the level of the ootype to form a short duct which continues mediad on the dorsal side of the body. Near the median line the common ducts from the two sides bend ventrad and unite to form the vitelline receptacle. The uterus passes backward in coiled masses almost to the posterior end of the body and then forward in the same manner until it reaches the metraterm. There is a strong tendency for the descending limb of the uterus to occupy the right, and the ascending limb the left, side of the body. The metraterm is on the left and the cirrus sac on the right side.

The eggs (Fig. 3) are oval, thick-shelled, and provided with a conspicuous operculum. Mature eggs vary from 0.028–0.033 mm. in length and from 0.016–0.021 mm. in width. Young eggs tend to be more spherical and the shells are lighter in color.

THE ASEXUAL GENERATIONS

A. *Primary Sporocyst*

Many attempts to infect *H. antrosa* have been unsuccessful and the factors which determine whether or not infection will be established are yet unknown. In 1935, however, experiments were continued and further information concerning the stages in the snail was secured. Eggs of *P. parva* were placed in containers with laboratory raised specimens of *H. antrosa* and at intervals the snails were removed, killed, fixed and cut in serial sections. Examination of sections shows that the miracidia emerge from the eggs in the anterior part of the small intestine and penetrate the intestinal wall. They remain in the adjacent lymph spaces and transform into primary or mother sporocysts. Often the infection was heavy and in one section three small mother sporocysts were visible in one low power field of the compound microscope. The primary sporocysts do not migrate into the digestive gland of the snail. They increase in size as the germinal cells multiply and form germ balls of the secondary sporocyst generation. For about ten days the primary sporocyst is irregularly oval in shape, but later the increase in number of germ balls and in size of the daughter sporocysts causes such distention that it becomes a shapeless, thin-walled sac. There is a birth pore at one end of the primary sporocyst and the daughter sporocysts emerge through the pore and migrate by way of the lymph channels to the visceral portion of the snail.

B. *Secondary Sporocyst*

These sporocysts (Figs. 4, 5, 6) are oval to vermiform sacs, 0.3 to 0.88 mm. in length and 0.056 to 0.16 mm. in width. They are very numerous, associated in masses in the interlobular connective tissue and

lymph spaces of the digestive gland of the snail and in the peripheral sinuses near the body wall. They are colorless, thin-walled, and variable in shape. Young sporocysts (Figs. 4, 5) are irregular to oval, often with solid projections at one or both ends, and not infrequently dumb-bell shaped with a solid shaft near the middle. Within the cavity there are clusters of germinal cells and in more advanced stages, often from 4 to 10 cercariaform larvae. As the germinal masses develop, the cavity in the sporocyst increases in size and becomes filled with larvae. All stages of development from germ balls to tailed cercariae occur regularly in a single sporocyst. Cercariae were observed to emerge through the birth pore, situated at one end of the sporocyst. In the examination of serial sections of snails which had been infected for several months, a few sporocysts were found which contained only unorganized parenchymatous tissue. They may be old ones in which the germinal cells have been exhausted.

C. *Cercaria*

The cercariae escape from the sporocysts and migrate through the tissues toward the cephalic end of the snail. It is probable that they complete their development during this passage, although infrequently they become mature and encyst without leaving the sporocyst. They have been found encysted also in the tissues of the newts. Such cysts are infective; the larvae excyst and develop when fed to the snail. Ordinarily the cercariae emerge from the snail and swim about near the bottom of the container. They are active for about twenty-four hours and die if they do not find an intermediate host.

Naturally infected snails have been kept for ten months in the laboratory and continued to give off cercariae during all that time. Immediately after they are brought into the laboratory, the number of cercariae given off is very large. Several hundred may emerge from a single snail in twenty-four hours if the temperature is high. These cercariae differ in size and appearance and consist of both mature and immature larvae. The small, immature ones are less active and die soon after emergence. They do not penetrate into intermediate hosts and are not infective. After a week or two in the laboratory, the number of cercariae which emerge daily has diminished to 20–40 mature, active larvae, and they are produced at this rate for several months. The great number which emerge when the infected snail is brought into the laboratory is believed to be the result of a rise in temperature when the snails are isolated in small amounts of water. After the snail has become adjusted to the higher temperature, the number of cercariae liberated falls to the usual rate. This opinion is supported by data from snails experimentally infected in the laboratory. In such snails, the number

of cercariae that emerge never exceed 100 in twenty-four hours. At first they appear in small numbers, then for a few days as many as 50–100 per day, after which the number liberated becomes stabilized at 20–40 per twenty-four hours. All these larvae are mature and active.

The cercariae emerge both during daylight and in the dark. They respond positively to light and tend toward the brighter side of the container. The body is relatively large compared to the size of the tail and the larvae do not swim rapidly. In swimming, the body is held rigid, contracted and bent so that it is concave on the ventral side, while the tail is extended and lashes vigorously. These movements impel the cercaria slowly upward and forward in the water but it begins to sink as soon as the body relaxes and the tail ceases to beat. A recently emerged cercaria will start swimming again when it touches the bottom. After a time the swimming reaction is less vigorous and the larvae creep about on the bottom.

The body of the cercaria (Fig. 7) is oval, usually more pointed anteriorly, although the shape varies with the elongation and contraction of different regions. Average measurements of twenty-five living specimens give body length 0.13 to 0.25 mm. and width 0.058 to 0.1 mm. The tail is subterminal in attachment and 0.05 to 0.15 mm. in length. The acetabulum is situated slightly behind the middle of the body and in living specimens measures from 0.028 to 0.036 mm. in diameter. The cuticula is spined over the entire body, and the spines are conspicuous on the lining of the cavity of the acetabulum. In the dorsal portion of the oral sucker there is a stylet, 0.017 to 0.02 mm. in length. The shaft is hollow and at the base of the anterior cone there are dorsoventral and lateral protuberances. Fixed and stained specimens vary from 0.1 to 0.2 mm. in length, 0.035 to 0.07 mm. in width and 0.02 to 0.04 mm. in thickness. The tail is 0.05 to 0.1 mm. in length. The acetabulum is 0.025 to 0.03 mm. in diameter; the oral sucker 0.03 to 0.035 mm. in diameter.

The mouth is subterminal and the oral sucker in living specimens varies from 0.04 to 0.053 mm. in diameter. The average of twenty-five measurements is 0.047 mm. The pharynx is 0.016 to 0.02 mm. in diameter and situated about one-third of the distance from the oral sucker to the acetabulum. The commissure of the central nervous system extends across the dorsal wall of the prepharynx. The alimentary tract bifurcates immediately in front of the acetabulum and the ceca extend in an arc around the anterolateral face of the acetabulum.

In no instance was it possible to observe all parts of the excretory system in a cercaria, but from many observations it is apparent that the excretory system at this stage is the same as that described for the recently excysted metacercaria and young worms.

The number and arrangement of the unicellular penetration and cystogenous glands are very difficult to determine. By long continued observation and the use of many vital dyes the outlines of the cells and ducts were traced. On either side there are two large penetration glands with finely granular cytoplasm, situated anterolaterally to the acetabulum. Behind them, on either side there are three penetration glands which have larger granules in the cytoplasm. The ducts from the penetration glands pass forward as shown in Fig. 7 and open at the sides of the stylet. When the cercaria is subjected to pressure under a cover glass the contents of the gland cells are forced into the ducts and pass forward. As the material passes along, the course of the duct may be traced. After long continued pressure the gland cells become more or less emptied and their secretion accumulates in the anterior portion of the ducts. The cystogenous glands are scattered about over the periphery of the body from the level of the nervous commissure almost to the posterior end of the body. These glands have large nuclei and the cytoplasm is stained by natural red.

D. *Metacercaria*

The cercariae penetrate actively into the second intermediate host, and entrance is undoubtedly facilitated by the activity of the stylet and secretions from the penetration glands. These glands are not completely emptied during the process, however, and may be recognized in recently encysted worms. Metacercariae have been recovered from dobson fly larvae and from various species of snails. The cysts are easily liberated from the surrounding tissues and may lie free in the lymph spaces of the host. The capsule is membranous and apparently it is formed by material from the cystogenous glands of the parasite. There is no outer fibrous coat deposited by the connective tissue of the host. The metacercaria entirely fills the cavity of the cyst.

The cysts are spherical to oval and have an average measurement of 0.12 mm. by 0.098 mm. They do not increase in size and those recovered one day after experimental infection are as large as others removed at the end of four weeks. Meanwhile the worm, although it may undergo some development, does not increase in size. As the larval structures regress, the metacercaria becomes clearer and more transparent. Soon after encystment the stylet is extruded and usually it can be observed lying free in the cyst. The excretory vesicle becomes filled with granular concretions and appears as a conspicuous dark spot when viewed with low magnification. Although thin and flexible, the cyst wall is very tough. It may be ruptured by pressure but in such instances the broken edges of the cyst usually cut and tear the worm as it escapes. In the intestine of the newt, although the cyst is not digested, it splits on one

side and the young worm emerges without injury. Since there is little development in the metacercarial stage, the second intermediate host serves primarily as a transfer agent.

DEVELOPMENT IN THE FINAL HOST

The course and rate of development in the final host have been traced by the removal of worms after various intervals in the intestine of the newt. Soon after excystment the concretionary granules are expelled from the excretory vesicle. A specimen recovered the second day after ingestion is shown in Fig. 8. The worm is no larger than the body of a cercaria and very little development has occurred. Compare figures 7 and 8, drawn at the same magnification. A specimen recovered twelve days after ingestion is shown in Fig. 9. It measures 0.85 mm. in length. The organs are clearly outlined although the female reproductive system is not yet mature. A specimen recovered eighteen days after ingestion is shown in Fig. 10. It is 1 mm. in length and all of the organs are actively functional.

DISCUSSION

The validity of the generic name *Plagitura* is doubtful. Price (1930) after comparing the type specimens and description of *P. salamandra* Holl, 1928, with the description of *Mandistomum occultum* Stafford, 1905, announced the identity of the two species.

Harwood (1932) reported specimens which he identified as *Manodistomum occultum* Stafford from *Triturus viridescens* taken at Elizabethtown, New York, and *T. meridionalis* at Houston, Texas. The specimen figured measures about 2 mm. in length and is not fully mature. It is about twice the size of a correspondingly developed specimen of *P. parva* and almost certainly belongs to the species described by Holl as *P. salamandra*. Since he could find no important differences between *Manodistomum occultum* Stafford and *Plagitura salamandra* Holl, Harwood agreed with Price in the identity of the two species and the suppression of *Plagitura* as a generic name. Furthermore, Harwood compared *Zeugorchis aequatus* with *Manodistomum occultum* and concluded that they are congeneric. Accordingly, he suppressed the genue *Zeugorchis* and transferred the species of that genus to *Manodistomum*.

There are cogent reasons for doubting the identity of *Plagitura* and *Zeugorchis* and it is difficult to determine which, if either, is to be suppressed as a synonym of *Manodistomum*. Data afforded by life history studies are of much significance in taxonomic considerations and since the life cycles of species belonging to both *Zeugorchis* and *Plagitura* are known, the developmental features of these and related species must be regarded in any discussion of the problem.

Sumwalt (1926) described *Zeugorchis syntomentera* from *Thamnophis sirtalis* and *T. ordinoides* collected on the Pacific coast. This species is so similar to *Z. aequatus* that Sumwalt expressed the possibility of their identity. The life history of *Z. syntomentera* was described by Ingles (1933). *Physa gyrina* serves as the first intermediate host. Ingles stated, "The cercariae of *Z. syntomentera* penetrate the skin and become metacariae in the tadpoles of *Hyla regilla, Rana aurora,* and even in the western newt, *Triturus torosus.*"

In an earlier paper, McCoy (1928) reported life history studies of four species which are closely related to *Z. syntomentera.* He stated, "Ova from specimens of *Renifer kansensis* Crow 1913, *Dasymetra conferta* Nicoll 1911, and *Pneumatophilus variabilis* (Leidy) Odhner 1910, obtained from snakes in the vicinity of St. Louis, were used to infest laboratory bred specimens of *Physa integra* and the cercariae of these three forms were obtained. The cercariae encysted in tadpoles but the entire life cycle was not completed." . . . "In addition, *Cercaria ramonae* sp. nov., from *Physa integra* was found to encyst in tadpoles and subsequently develop in snakes into young worms which most probably belong to the genus *Renifer.*"

Talbot (1933) has traced the life cycles of *Lechriorchis primus* Stafford, *L. tygarti* Talbot, and *Caudorchis eurinus* Talbot. He studied the miracidia of six species in the subfamily Reniferinae and reported that "no structural differences could be found whereby they could be distinguished." In all but one case the miracidia emerge only after the eggs have been ingested by a snail. Talbot pointed out that, "at present we have adequate information on the cercaria stage of seven species of six genera belonging to the Reniferinae. In all of these the structure of the cercaria shows a striking similarity." The cercariae have the same excretory pattern, and all develop in species of *Physella.* In every instance the cercariae penetrate into tadpoles and encyst. Later they develop to maturity in the lungs and oronasal cavities of snakes. According to Talbot, "All of these species show a remarkable uniformity in the course of their life cycles and in the structure of their larval stages, a fact which indicates the importance of these characters in any adequate definition of this subfamily."

More recently Byrd (1935) has described the life history of *Renifer aniarum* and *Dasymetra villicaeca.* The intra-molluscan generations of both species occur in the snail, *Physa helei,* and the cercariae encyst in tadpoles. From the studies of McCoy, Ingles, Talbot and Byrd it appears that all members of the subfamily Reniferinae typically attain sexual maturity in reptilian hosts, that their asexual stages develop in species of the genus *Physa,* and that the metacercariae occur in tadpoles.

The experimental demonstration of the life cycles of *Zeugorchis syn-*

tomentera and *Plagitura parva* indicates that the two species are not congeneric. *Zeugorchis* is a parasite of the lungs and oronasal cavities of snakes, asexual multiplication occurs in species of *Physella,* and the cercariae encyst in tadpoles. *Plagitura,* on the other hand, is an intestinal parasite of *Triturus,* asexual multiplication occurs in species of *Helisoma,* and the cercariae encyst in various invertebrates. They will not penetrate into tadpoles or newts. Both *Zeugorchis* and *Plagitura* have been regarded as synonyms of *Manodistomum* and it is a question which if either is congeneric with *M. occultum.* The problem is created by the inadequate description of the latter species.

As a basis for discussion, Stafford's (1905) account is given in full. He stated,

"65) *Manodistomum occultum.* From *Diemyctylus viridescens* Raf. (Newt). *Rana virescens* Kalm. (Common Frog, Green Frog).

New genus and species: μανός rare; occultus, obscure. Length (permanent mount) 1,15, breadth 0,399. Mouth sucker 0,184 × 0,189. Ventral sucker small, about 0,107, in center of body. Pharynx small, immediately behind mouth sucker. Oesophagus 0,23 long, narrow. Caeca twice as long as oesophagus, falling some distance short of posterior end. Excretory bladder broad and long, forking at the midde of the length of the coeca not far behind the ventral sucker. Immature, but parts of the genital organs represented by rudiments. Testes small, globular bodies, in a line with but behind the ends of the coeca, dividing the space between these and the end of the worm. They are lateral and parallel and give off fine ducts anteriorly. Ovary crowded between ventral sucker and the fork of the excretory bladder. Genital pore in the short space between the ventral sucker and the fork of the intestine, with the end organs of the genital ducts faintly outlined. No recognizable vitellaria.

The worm thus described I have come upon but once in a newt and I am unable to give its exact habitat. It appears, however, to be the same as one I have already reported (Zool. Anz. Bd. XXV. Nr. 673/674. 1902. p. 482. Nr. 13) although more mature. The description given there (with the decimals corrected) reads: 'Encapsulated on muscles in posterior part of body cavity of *Rana virescens* at Canso N. S. Length (living) 1,3, breadth 0,6, pharynx 0,12, oesophagus 0,15, mouth sucker 0,21, ventral sucker 0,12 in middle of animal. Coeca to half way between ventral sucker and posterior end. Skin with spines. Immature.' The shape and size of the worm, the relative size and position of the suckers, the intestinal system and the excretory system agree. In the specimens from the frog I was unable to recognize rudiments of the genital system.

These worms bear many resemblances to Nr. 86 (described later in the same paper as *Zeugorchis aequatus* new genus and species) from the snake of which indeed they may be the young."

What is *Manodistomum occultum*? The description of the species was based on material from both the newt and frog; measurements of one specimen from each host were given. Stafford regarded these specimens as specifically identical. The specimen from the frog was encysted, the habitat of the one from the newt was unknown. If the specimen from the newt had been encysted previously in that host, it may be specifically identical with the one from the frog. In the dissection of the newt it is possible that Stafford ruptured a cyst and liberated the specimen whose habitat he was unable to give. Or, if the dissection was delayed for some time and decomposition of the tissue had begun, the worm may have emerged spontaneously. Indeed, Stafford's suspicion that both were larvae of *Zeugorchis* may be correct. On the other hand, the specimen from the newt may not have been encysted in that host, in which event it could not be identical with the specimens from the frog. If it came from the intestine, which is not improbable, it may be identical with *Plagitura salamandra*. It is not *P. parva* since the worm was 1.15 mm. long and the oral sucker measured 0.184 by 0.189 mm. In *P. parva* the oral sucker rarely if ever attains that size and specimens over 1 mm. in length are sexually mature. From available data, one can not determine with certainty whether the two forms described by Stafford are identical or not. The specimens from the frog may be larvae of *Zeugorchis aequatus* or some related species. It is reasonably certain that they are not larvae of *Plagitura*. The specimen from the newt may have been a larva of *Zeugorchis* or a young specimen of *Plagitura salamandra*. If Stafford's description of *Manodistomum occultum* was based on material of two species, any subsequent author may reserve the name for one of them and rename the other species. Since at present it is impossible to determine whether Stafford had one or more species, it is impossible to identify or recognize *M. occultum*.

The paper by Price (1930) announcing the identity of *Manodistomum occultum* and *Plagitura salamandra* was written before the life history of *Zeugorchis* was discovered. He assumed the description of *M. occultum* was based primarily on the specimen from *T. viridescens* and that the specimen came from the intestine. Neither assumption appears to be supported by adequate evidence. Price suppressed *Plagitura* as a synonym of *Manodistomum* on the basis of similarity in the position of the testes with respect to the termination of the intestinal ceca and the identity of hosts. Neither of these observations necessarily supports his conclusion, since the same features are common to both *Plagitura* and *Zeugorchis*. In the latter genus the testes have the same relation to the ceca (see figure in Sumwalt, 1926) and the larvae may occur in *Triturus*.

While Price may be correct in his suspicion that Holl's specimens are identical with the one Stafford found in a newt, the experimental demon-

stration of the life cycles of *Zeugorchis syntomentera* and *Plagitura parva* invalidates Harwood's opinion that both *Plagitura* and *Zeugorchis* are synonyms of *Manodistomum*. It would be difficult to maintain that the species from the newt described by Holl and the writer are congeneric with those from snakes that have been placed in the genus *Zeugorchis*.

According to the International Rules of Zoological Nomenclature *Manodistomum* is a valid name. It was published and accompanied by a description of specimens as provided in Article 25. It is sustained by the Law of Priority since Article 27 provides that a name is retained when any stage in the life cycle is named before the adult. Since it is impossible at the present time to recognize specimens of *M. occultum* or to determine whether either *Zeugorchis* or *Plagitura* is synonymous with *Manodistomum,* all three generic names should be retained. The question of synonymy must await further evidence.

SUMMARY

Data are presented to support the specific identity of *Plagitura parva* and it is compared with *P. salamandra*. The life history of the former species is traced and all stages in the cycle are described. The validity of the generic name *Plagitura* and the relationships between *Plagitura, Manodistomum* and *Zeugorchis* are discussed.

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Explanation of Figures

Abbreviations

ac—acetabulum
ad—anterior excretory collecting duct
cd—common excretory collecting duct
ce—developing cercaria
cs—cirrus sac
ev—excretory vesicle
fc—flame cell
gm—germ mass
gp—genital pore
in—intestine
mt—metraterm
ov—ovary
pd—posterior excretory collecting duct
pg—penetration glands
sv—seminal vesicle
ts—testis
ut—uterus
vd—anterior vitelline collecting duct
vt—vitellaria

Plate 1

Fig. 1. *Plagitura salamandra,* co-type specimen from Holl, ventral view, ×48.
Fig. 2. *P. parva* type specimen, dorsal view, ×48.
Fig. 3. *P. parva,* egg, ×750.

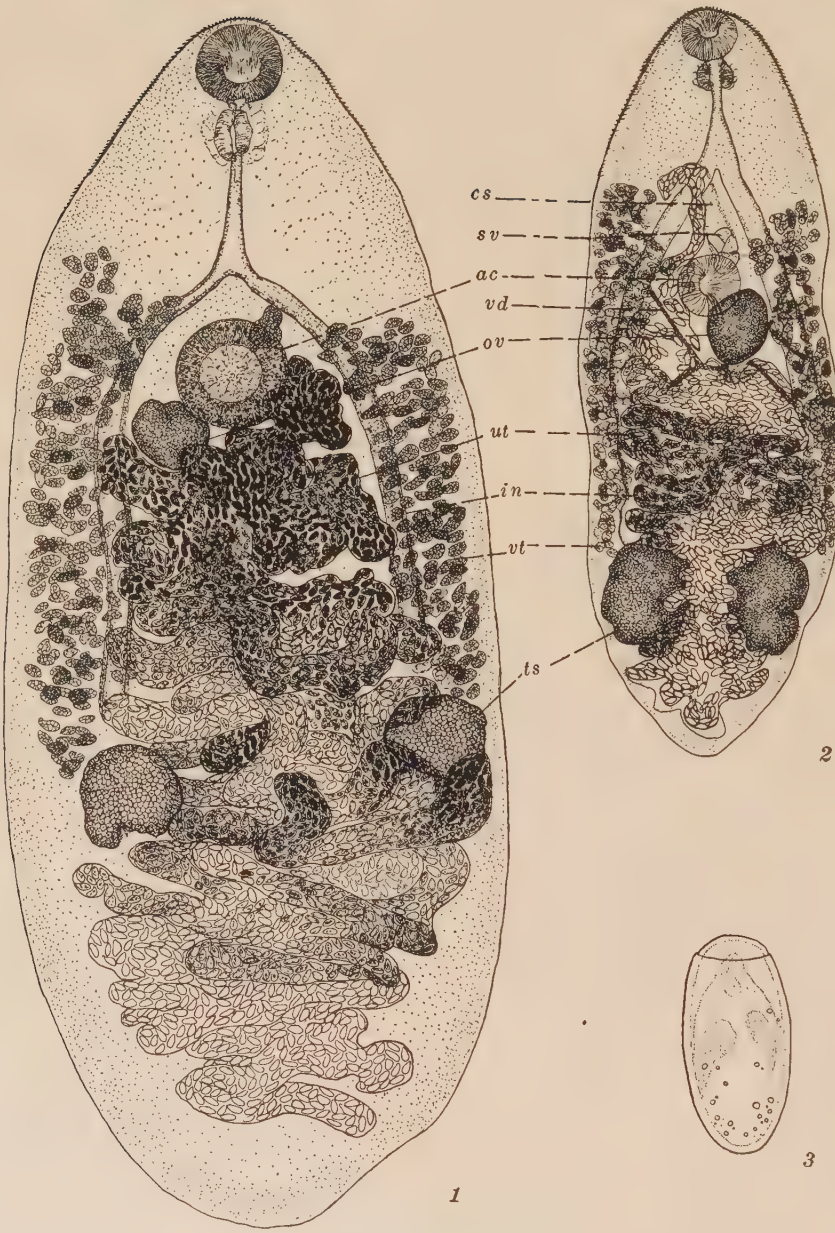
Plate 2

Fig. 4. *P. parva,* outline sketches of daughter sporocysts.
Fig. 5. *P. parva,* young daughter sporocyst, ×147.
Fig. 6. *P. parva,* old daughter sporocyst, ×147.
Fig. 7. *P. parva,* cercaria, outline from fixed stained specimen, details from many living larvae, ventral view, ×530.

Plate 3

Fig. 8. *P. parva,* excysted metacercaria recovered from newt 40 hours after feeding, from stained whole mount, ×530.
Fig. 9. *P. parva,* from newt, 12 days after feeding, dorsal view, ×90.
Fig. 10. *P. parva,* from newt, 18 days after feeding, ventral view, ×90. *sv,* in this figure indicates the seminal receptacle and not the seminal vesicle as in Fig. 2.
Fig. 11. *P. parva,* diagram of excretory system of sexually immature worm.

PLATE I



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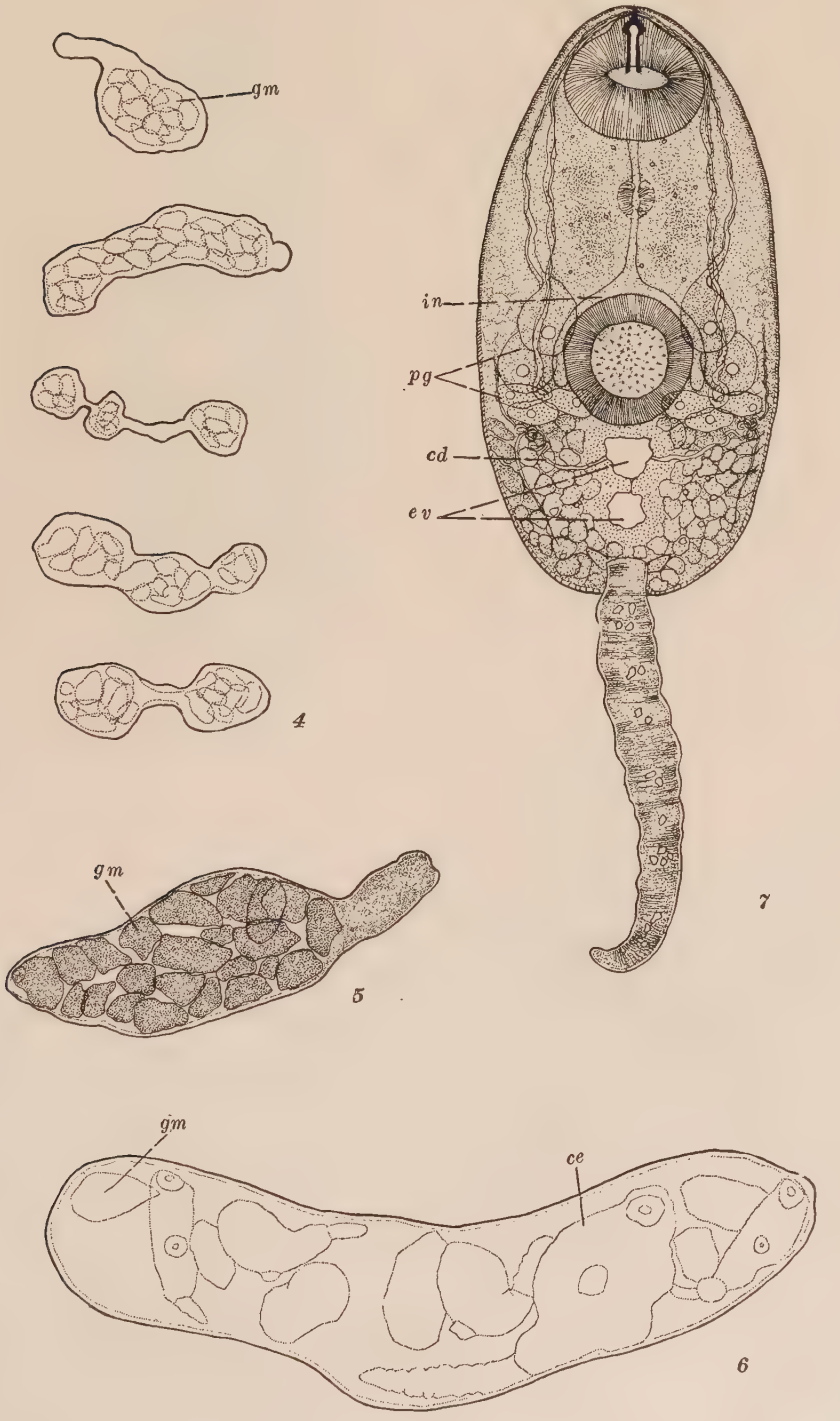
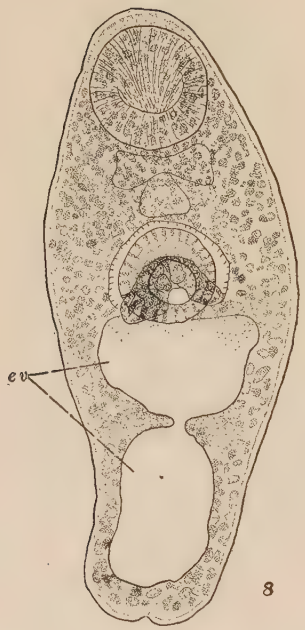


PLATE III



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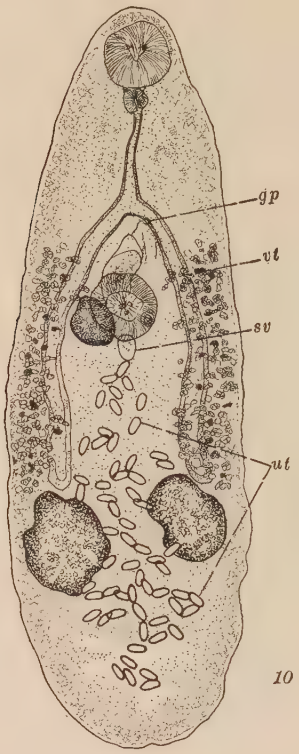

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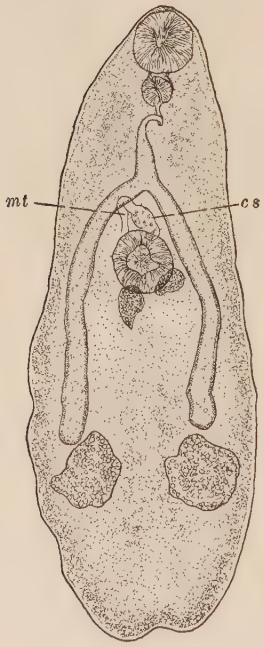

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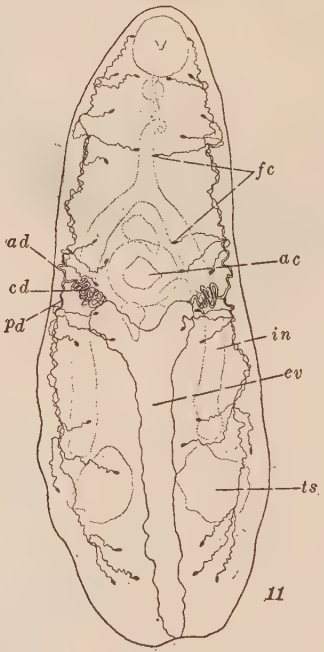

11

NEW SPOROCYST OF *LEUCOCHLORIDIUM* FROM LOUISIANA[1]

CARL GOWER

INTRODUCTION

The first larval stage of the Genus *Leucochloridium* from the United States was described by Magath in 1920. Magath gives a very excellent account of the history of the Genus. Carus created the Genus in 1835, and since then a number of species have been described in Europe. Wesenberg-Lund is the outstanding present-day European worker, and has recently published (1931) a comprehensive paper on *Leucochloridium paradoxum.* In this country, aside from the already mentioned work of Magath, Woodhead (1935) has described four different sporocysts. These were taken from snails collected in the Lake Erie marshes about eight miles north of Toledo, Ohio. McIntosh (1932) has been the sole investigator to work on the adults of the Genus in this country, having described twelve species from North America. Since one of Woodhead's sporocysts is identical with the one described by Magath, there have been four different sporocysts described from North America, and twelve species of adults.

The author wishes to express his appreciation for the help received from Dr. H. J. Bennett and Dr. E. L. Miller of Louisiana State University, and to Dr. A. E. Woodhead of the University of Michigan.

LOCATION

On April 13, 1934, while collecting along the margin of a small lake about twenty miles north of Baton Rouge, Louisiana, in the northwest part of East Baton Rouge Parish, the author picked up a single specimen of *Succinia retusa* Say parasitized by a large sporocyst of *Leucochloridium.* As it was almost dark, only a short search was made for other infected snails at this time. On the next day a trip to the same spot yielded thirty infected specimens, and on a subsequent trip a week later sixteen more were collected. All of the infected snails were *Succinia retusa.* These infected snails were found at only one location, and although an extended search was not made, parasitized snails were certainly not common in the general vicinity of Baton Rouge. So far as it has been possible to determine, no one has previously found either larval or adult forms of this parasite in Louisiana.

The place of collection was a small lake near Port Hudson, Louisiana. The lake is very near the Mississippi River, and at times is flooded by

[1] Contribution from the Zoology Department of Louisiana State University.

backwater. As the general level of the river was low in 1934, this lake was not flooded that spring. The lake covered about twenty acres when the collections were made. The water was comparatively shallow, averaging not more than five or six feet deep. For the most part Buttonbush, *Cephalanthus occidentalis,* and Black Willow, *Salix niger,* lined the shore. There was practically no emergent vegetation in the water, but a great amount of submerged vegetation consisting chiefly of Hornwort, *Ceratophyllum.* At the time of collection the wind had been blowing from the southeast for several days, and masses of this vegetation were piled along the north and northwest shores. All of the infected snails were collected along a strip of beach at the northwest corner of the lake where the shrubby vegetation did not approach the water line. The infected area was not over two hundred yards long, and extended from the water line not over ten feet. The same species of snails along with some five other species were picked up around the entire lake, but none of those collected away from the above mentioned locality showed any sign of infection. Many of these were brought into the laboratory and kept for a while to find out if they would develop a noticeable cyst, but the results were negative in every case. It must be stated that the above mentioned focus of infection was the particular part of the lake where a great many domestic animals came to drink, and probably was used a great deal by birds for drinking and bathing. This fact might account for the lack of infection at other points in the lake. The fact that this beach was exposed to sunlight for a long period every day is also of significance.

DESCRIPTION OF THE BROOD-SAC

Only one type of brood-sac (the cysts are within the "brook-sac") was found. The following description is taken from a mature sac of average size and coloration (Fig. 1). The general ground color of the sac and stalk was yellowish-white, closely approaching flesh color. The lower two-thirds of the brood-sac (but not the stalk) was marked with crosswise, broken, irregular streaks of brownish-green. Above this area was a broad band of bright red which appeared to be streaked with white when the sac pulsated. Equally spaced from the cap markings, and with intervening areas of white, were two bands of yellowish-green. The cap was marked by orange-brown with a center of chestnut red. The sac described above had the following measurements: total length 14 mm., length of sac 9 mm., average width 3 mm., and width of tube at base .5 mm. In immature sacs these areas of pigmentation were marked by bands of pale green. In two cases immature sacs pulsating in the tentacles of snails were noted.

In general sacs taken from any particular snail appeared to mature in

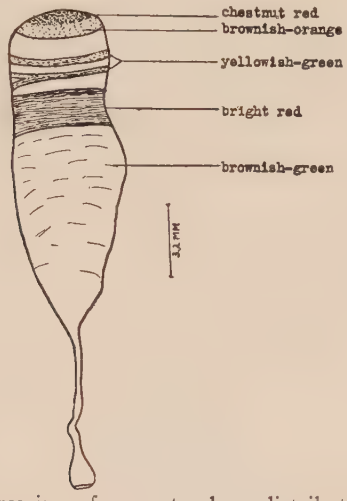

FIG. 1. Line drawing of a sac to show distribution of pigment.

pairs. There was one exception to this rule, when a snail was found with three mature sacs. In this case, when the three sacs were pulsating at the same time, the third sac took a position over the radula of the snail, as was Woodhead's observation in a similar case. When all three sacs were pulsating at the same time the entire anterior part of the snail seemed to be completely filled. An attempt to keep alive 20 infected snails in a tub filled with moistened earth and decaying plants failed, as most of the snails died in less than a week.

DISCUSSION

The only locations from which larval or adult forms of this trematode have been previously reported are Iowa, Michigan, Ohio, and possibly Indiana. This record comes from a point at least 1000 miles south of any previously recorded location. Due to the migratory habit of birds this fact is less impressive, for the same species might occur in Michigan. The bird harboring the adult of this parasite is yet to be found, but will probably be some passerine species. The author attempted to infect a female Mallard duck with this parasite by feeding the entire infected snail (shell removed) in a gelatin capsule. The duck was kept in a large cage in the laboratory, and fed two snails each week for three weeks. Four days after the last feeding the bird was killed, but a careful autopsy failed to reveal any trematodes. Later a male Cardinal was kept in the laboratory, and fed two snails. The bird died suddenly six days later. An autopsy was performed and eight small trematodes found which as yet have not been identified as *Leucochloridium*.

In comparing the sporocyst described in this paper with those described by Magath and Woodhead, it is noted that all of them differ in

color. None of the brood-sacs previously described have had so many different colors. The pigment is entirely in bands, and there is no semblance of striping. Also, most of the other sacs are described as being more or less pointed at both ends, whereas, all of the sacs observed in this case were rounded distally, and gave no indication of being pointed.

The morphology of the encysted cercaria (Fig. 2) differs from

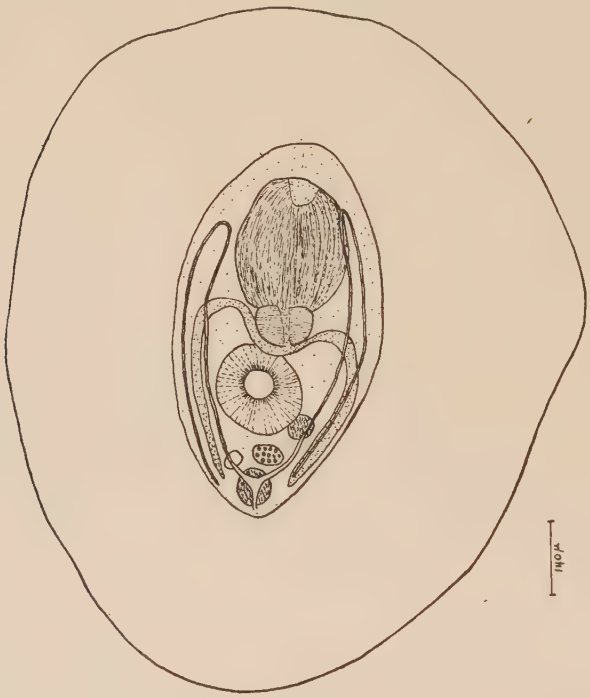

FIG. 2. Camera-lucida drawing of an encysted cercaria showing general morphology and relative size of cyst.

Magath's drawing of *Leucochloridium problematicum*. The enveloping cyst is much larger in this case, and the oral sucker is larger proportionally, being almost twice as large as the acetabulum.

SUMMARY

1. Notes on the larval stage of a *Leucochloridium* differing in coloration from any previously described from North America are presented.

2. The location record is a new one for the Genus in North America.

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OBSERVATIONS BASED ON WEEKLY PARASITE EGG COUNTS ON FECES OF LAMBS AND YEARLING SHEEP*

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As a phase of the study of intestinal parasites in Montana sheep, two series of weekly fecal examinations of lambs and yearlings have been made. The sheep used in this work were managed under conditions comparable to those under which farm flocks are handled in Montana, as contrasted with range conditions. They were part of a small flock of about 150 sheep carried by the Veterinary Research Laboratory at the Montana Experiment Station. The ewes which were the foundation of this flock were range sheep. Shed lambing in March and April is practiced. During the summer the sheep are run in a small valley pasture, and fed hay without any grazing for five winter months, December to April inclusive.

RESULTS OF EGG COUNTS IN LAMBS

The first series of fecal examinations was made on twelve lambs during the first eight months of life. These lambs were born between April 4 and April 16, and the first fecal examination was made on April 29, when the oldest lamb was twenty-five days and the youngest thirteen days old. Weekly examinations were made thereafter until December 28.

Eight weekly fecal examinations were also made on three lambs born between May 13 and May 18, the first sample being taken on May 26.

The method of making the counts was the Lane "direct centrifugal flotation" method as described by Stoll.[1] The fecal sample was taken from the rectum of the lamb. Where possible, 10 grams or more were collected, but in the very young lambs it was often impossible to obtain that amount from the rectum. A 10-gram sample was weighed out and placed in a flask containing 300 cc. of water. The feces were broken up with a glass rod, and the flask was allowed to stand about twenty-four hours to further macerate the material. At the end of that time the flask was thoroughly shaken to make an even suspension of the fecal material. A 10 cc. sample was withdrawn and placed in a centrifuge tube. The samples were centrifuged at 1000 r. p. m. for one minute, after which the supernatant fluid was poured off. At this point our method departed

* Contribution from Montana State College, Agricultural Experiment Station. Paper No. 70, Journal Series.

[1] Stoll, N. R. 1930.—Methods of counting nematode ova in sheep dung. Jour. Parasitol., 22: 116.

from the method described by Stoll. In his method the tube was then filled with saturated sodium chloride solution. In our dry atmosphere, crystallization of the salt gave so much trouble that a sugar solution (130 g. cane sugar in 100 cc. water) was substituted. This change probably resulted in somewhat lower counts due to the viscosity of the solution. After refilling the tube level with the lip with the sugar solution, an 18 mm. square cover glass was placed over the tube so that the fluid just covered the under surface of the cover without overflowing. The samples were again centrifugalized at 1000 r. p. m. for one minute. The cover slip was then carefully lifted from the tube and transferred to a slide, carrying a film of the sugar solution containing the parasite eggs. All the eggs in the preparation were then counted. Multiplying this count by three gave the number of eggs counted per gram of feces, as each 10 cc. sample of the original suspension represented one-third gram. This number was, of course, somewhat less than the actual number. The eggs observed were those of *Ostertagia circumcincta, Nematodirus spathiger, Trichuris ovis,* and *Moniezia expansa.* Those recorded as *Ostertagia* may have included a few *Haemonchus* and *Trichostrongylus.* Records were also kept on the counts of coccidial oocysts. The data on coccidia will be discussed in another report.

Ostertagia eggs were first observed on May 19 in five lambs, the youngest of which was 42 days old. In eleven of the twelve lambs born in April, the first *Ostertagia* eggs appeared 42 to 58 days after the birth of the lambs, the average age being 48 days. *Nematodirus* eggs first appeared in one lamb on May 19, when it was 44 days old. In eleven April lambs, *Nematodirus* eggs first appeared at from 44 to 64 days after birth, the average being 56 days. In the three lambs born in May, nematode eggs appeared somewhat earlier. The first *Ostertagia* eggs were observed at 22 days, and the first *Nematodirus* at 27 days.

Trichuris eggs were present at times in the feces of all the lambs. The earliest date on which *Trichuris* eggs were observed was June 14, in two lambs which were 68 days old. Beginning about July 20, *Trichuris* eggs were present quite constantly in the feces of all the lambs, but for the most part in small numbers. The largest number recorded was 294 per gram. Three lambs which had shown the presence of *Trichuris* were negative for eggs of this parasite during the months of November and December.

Moniezia eggs were observed in the feces of nine lambs. The earliest date on which *Moniezia* eggs were found was August 24, in a lamb 141 days old. In the nine lambs, the average age at which *Moniezia* eggs appeared was 172 days.

Curves were plotted for each lamb, showing the number of eggs per gram of feces of *Ostertagia, Nematodirus,* and *Moniezia.* Chart 1

shows curves based on the average weekly count of the twelve lambs for each species of parasite. The first eggs were observed on May 19. The *Ostertagia* curve shows a rather gradual rise up to 87 on July 27. Between July 27 and August 10 there is a sharp rise, the average count reaching 268 on August 10. The number dropped off rather sharply to 92 on September 7. After a slight secondary rise in September and October, the number of *Ostertagia* eggs dropped gradually to 59 on December 21. On December 28, the date of the last examination of this series, there was a rise to 100. The maximum number of *Ostertagia* eggs counted was 711 in Lamb 136 on August 10.

The curve for *Nematodirus* showed a similar form, the number of eggs reaching the high point of 104 on August 3. The number dropped back to 17 on September 7. After a slight secondary rise in September and October, the *Nematodirus* egg count remained between 6 and 14 from October 19 to December 21, after which there was an increase to 25 on December 28.

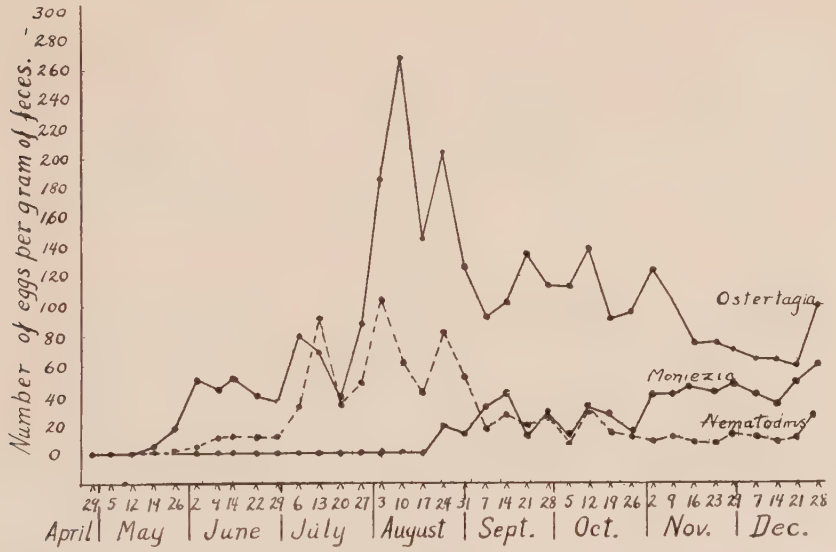

CHART 1. Graphs showing average weekly fecal egg counts for twelve lambs.

The *Moniezia* curve showed a gradual rise from August 24, when the first *Moniezia* eggs were seen, to a count of 60 per gram on December 28.

RESULTS OF EGG COUNTS IN YEARLINGS

A series of weekly egg counts was made on four yearling sheep, covering a period of eleven months. The first examination was made on January 20, when the sheep were nine months old. On June 6, four more sheep of similar age were added to the group, making a total of

eight for the latter half of the year. Chart 2 shows curves based on the average weekly counts for each species, the time covered by the chart being from April 22 to December 31. The average counts previous to April 22 were computed but were not included in the chart.

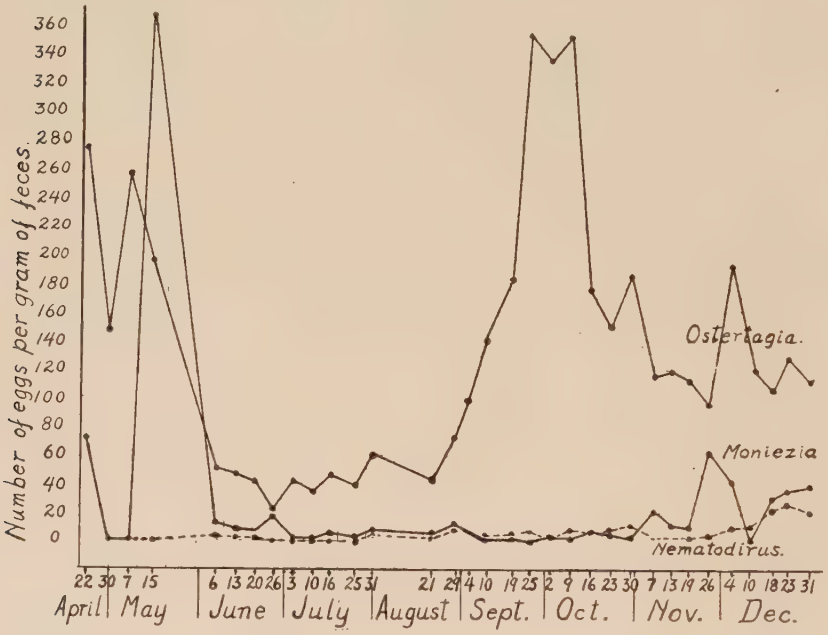

CHART 2. Graphs showing average weekly fecal egg counts of eight yearling sheep.

The number of *Ostertagia* eggs showed a rise from 406 on January 20 to 587 on January 22. The counts varied between approximately 400 and 600 until March 13, after which there was a sharp drop to 80 per gram on March 20. There followed a gradual rise to 274 on April 22. Referring to chart 2, it will be seen that after April 22 the *Ostertagia* egg count dropped until the low point of 23 was reached on June 26. The counts remained low until August 21, after which there was a rather sharp rise to a high point of 353 on September 25. After October 9 there was a rapid decline to 96 on November 26, with little change to the date of the last examination on December 31.

The *Nematodirus* count was low in January and February, but rose to a high point of 288 on March 2, falling back to zero on March 13. Through the remainder of the year the *Nematodirus* count was very low, showing a slight increase during December.

The *Moniezia* egg count showed wide variations from January to June, with three high points in February, April, and May. The highest average count was 370 on April 9. Between May 15 and June 6 the

Moniezia count dropped from 366 to 13, and remained below 20 until November 7. During November and December there was a slight increase in the number of *Moniezia* eggs.

AUTOPSY FINDINGS

In the cases of eight of the twelve lambs in the first series, post-mortem examinations were made within seven months after the close of the period during which fecal examinations were made. These were chiefly of value in checking on the species to which eggs had been referred in making fecal examinations. This was of particular interest in differentiating between *Haemonchus* and *Ostertagia* eggs. In routine egg counting it is difficult to distinguish with certainty between these eggs. As previous work had established the fact that *Ostertagia* is the predominant nematode in the abomasum in Montana sheep, eggs of that type were recorded as *Ostertagia.* The autopsies justified this assumption, as six of the eight lambs killed were entirely free of *Haemonchus,* and the other two stomachs contained a total of three individuals.

Careful examination of the abomasum and the entire small intestine was made in six cases. In three of these lambs *Trichostrongylus* was found in small numbers. Eggs of these nematodes were not differentiated in the routine fecal counts. The number of *Ostertagia* found in these six cases varied from 40 to 1500, and the number of *Nematodirus* varied from none to 5000.

DISCUSSION

This report records observations which have application to two subjects—the seasonal cycle of egg production of *Ostertagia, Nematodirus,* and *Moniezia,* as indicated by fecal counts; and the age at which parasite eggs first appear in the feces of lambs.

As shown in chart 1, lambs born during the first two weeks of April showed the first parasite eggs in the feces on May 19. After July 20 there was an abrupt increase in nematode egg numbers to August 10, followed by a rather abrupt drop until September 7, after which there was no great variation, there being a gradual decrease to December 21. There was a slight increase in the counts made on December 28, indicating a possible tendency toward a January increase.

The observations on yearlings were not made on the same animals which were used for the lamb work, and the work was done during another year. The average counts for the yearlings show for *Ostertagia* a high point in January and February, dropping off sharply in March, and then more gradually until a low level was reached which persisted through June, July, and August. In September there was a sharp increase to a high point about October 1, with a drop during October. The

Nematodirus count was low in this group of sheep throughout the year, except for one high point in March. In September of the year preceding the January in which the yearling counts were made, there were mild clinical cases of parasitism in the bunch of lambs from which the eight sheep of the yearling group were selected. On September 9, fecal egg counts were made on twenty-seven lambs of this lot. The average *Ostertagia* count was 636, with a maximum individual count of 5916. No counts were made between September and January, when the average count was 587. We have no information as to whether there was a drop between September and January.

As would be expected, the average curves for both the lambs and the yearlings showed a maximum production of *Ostertagia* eggs for a short period during the summer months. The high point for the lambs was in August, while for the yearlings the high point was in September, the counts being very low through June, July, and part of August. These summer peaks are undoubtedly due to rapid infestation during the warm weather when conditions are favorable to development of infective larvae. The earlier appearance of the high point in the lamb group may be due to the greater susceptibility of the young lambs, but the effect of different weather conditions and pasture conditions may also have been a factor in the lag shown in the yearling curve.

The high counts in the yearling group in January and February were probably a continuation of the high counts recorded in September, rather than a new peak. Under the climatic conditions of the Montana Station, there is little opportunity for reinfestation between November 1 and May 1. The curve indicates that egg production by the females, which established themselves in the sheep during the preceding summer, continued high until March 13, after which these females gradually dropped off in egg production to a fairly constant low level through June, July, and August. During these months reinfestation took place until in the latter part of September and early October there was fairly high egg production. This apparently slow reinfestation and the abrupt drop back to relatively low counts in November may indicate an immune condition in these yearlings, as compared with the susceptibility of lambs.

In considering the question of the earliest age at which parasite eggs appear in the feces of lambs, it is evident that the date of birth of the lambs is an important factor. In the group of lambs studied, twelve were born between April 4 and April 16. During April and the first thirteen days of May, cool weather prevailed. There were only nine days when the maximum temperature exceeded 60° F., with the minimum daily temperature below 40° for the whole period. Seven of the nine warm days were consecutive days from April 22 to April 28, which was probably the period during which the first infective larvae were

picked up by this group of lambs, as in eight of these lambs the first eggs were recorded on May 19 and May 26. Due to cool weather, this group of lambs, therefore, had no opportunity to become infested until they were at least fourteen days old.

The three May lambs were born on May 13 and May 18. The temperature record shows that from May 14 to May 31 there were only five days when the maximum temperature was below 60°, the lowest daily maximum being 54°. The temperature conditions were therefore favorable to the development of infective larvae from the time the lambs were born, and in one of these lambs the eggs of *Ostertagia* were found in the feces on the twenty-second day after birth.

SUMMARY

Weekly fecal parasite egg counts were made on a group of twelve lambs from birth in April to December 31, and on four yearling sheep from January to December. These counts were supplemented by a series of weekly counts on three lambs born in May, and by the addition of four sheep to the yearling group from June to December.

The eggs recorded were those of *Ostertagia circumcincta, Nematodirus spathiger, Moniezia expansa,* and *Trichuris ovis.*

Curves recording the average *Ostertagia* egg counts for each group showed in both groups a summer peak, reached in August for the lambs and in September for the yearlings. In each group there was a sharp drop during the month following the peak.

In the lambs, the earliest appearance of parasite eggs was at the age of 22 days, at which time *Ostertagia* eggs were found in one of the May lambs. *Nematodirus* eggs were observed in one case at 27 days. No *Moniezia* eggs were observed until 141 days after birth.

OBSERVATIONS ON CATTLE NEMATODE INFECTIONS, WITH A DEMONSTRATION OF THEIR SECONDARY TRANSMISSION TO GRAZING SHEEP

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While the gastro-intestinal nematodes of sheep have long been a subject of inquiry in many parts of the world, similar attention has not been given to the problem in the bovine host. This may be due in part to the rarity of troublesome nematode outbreaks in cattle, as well as to the apparently greater difficulty of studying their subclinical infections. The parasitic species in cattle, sheep and goats are largely identical, but whether they represent host specific strains or are freely cross-infective has likewise been a subject of but little inquiry.

Following the establishment of a new pasture designed primarily for a laboratory herd of cattle, observations have accumulated which permit orientation on certain aspects of the bovine problem. When the herd was begun, some care was taken to prevent extensive verminoses in the calves initiating it, but this attempt was not rigorous and a generous number of species later revealed their presence. While these helminthic infections have been only a subsidiary interest in the cattle, a fairly clear picture of the grade of infections which develop under such conditions has resulted. A group of sheep pastured with the cattle for two grazing seasons became infected with eight species of seven genera of the nematodes brought to the pasture by the cattle. This report also analyzes the status of flotation examinations of cattle dung and besides indicating the worm species involved in the natural experiment, gives additions from other bovine autopsies at this laboratory in comparison with three American check-lists.

METHODS

The course of infections in both sheep and cattle was followed by fecal examinations supplemented by autopsies.

The adaptation of a salt flotation method to produce reliable quantitative results with *Haemonchus contortus* eggs in sheep dung has been described (Stoll, 1930). (Lane in a reviewer's comment, Trop. Dis. Bull., 1930, *27*, p. 971, finds that this adaptation of his technique has "altered it in essential particulars." It seems accordingly inappropriate to refer to it as D. C. F.) In our experience this technique is effective for the eggs of other strongyloid worms of the ovine alimentary tract. It has been used without further modification for cattle examinations.

In evaluating its comparative use on cattle dung, three items have been scrutinized: (1) the efficiency of ova flotation from a single tube of water suspension containing one-half gram dung, (2) comparability of results of one such tube and others poured from the same suspension, and (3) the status of negatives. Pertinent, routinely collected data concerning eggs of strongyloid type, show:

1. In flotation from a given tube, nine-tenths or more of the eggs appear on the first coverslip. Of 20 tubes from different fecal suspensions with 1 to 29 eggs on first cover, all second covers were negative; in 5 of these tubes a third cover was run and it was also negative. In 12 tubes ranging 2 to 127 eggs on first cover, the second covers were positive; three carried to third covers showed respectively 9–2–0, 23–1–1, and 44–3–0 eggs. These twelve tubes totaled 488 eggs on first cover, 51 on second. Assuming all third covers would have been negative or nearly so, and totaling the above 32 tubes, there was first cover efficiency of 93 per cent. A more detailed analysis on flotation from sheep feces showed 87 per cent of *Haemonchus* eggs delivered on first covers, of hookworm eggs from human feces, 60 per cent (Stoll, 1930).

2. Flotation results from different tubes of the same bovine fecal suspension are substantially similar. When one tube was negative, of 19 duplicate tubes from the identical flasks, 16 were negative, 2 had 1 egg each, 1 had 3 eggs; of the negative duplicate tubes, 15 examined in quadruplicate showed 12 continuing negative, 3 others having 1–2 eggs each. Of 6 flasks demonstrating 1 egg per tube, five had 1 or 2 eggs on duplicate or triplicate tubes; in one case there were 5 eggs from a second tube. Of 19 other flasks with 2 to 301 eggs in the first tube examined, second tubes had 3 to 296 eggs. One flask showed 53, 52 and 55 eggs from three successive tubes, another 12, 9, 8 and 10 from four.

Further insight is obtained by comparing flotation examinations and either dilution counts, or larval yields after culture. Of 25 fecal samples from which both water suspensions for flotation examination and decinormal sodium hydroxide suspensions for dilution counts were made, 23 of the latter were negative, with the comparative flotations ranging from negative to 49 eggs. A single egg on dilution would have been equivalent to 100 by flotation. Two flasks with "numerous" eggs by flotation showed respectively 2 and 3 eggs by dilution.

Six specimens were cultured, and two with 39 and 98 eggs per gram had 13 per cent and 2 per cent larval yields respectively. Of four specimens negative by flotation, each of two cultured in 30 gram amounts was negative, a culture of 91 grams yielded 1 larva from a third specimen, a culture of 153 grams yielded 3 larvae from the fourth specimen.

This analysis indicates that when a given bovine fecal suspension is positive by flotation for strongyloid eggs, the yields of eggs from suc-

cessive flotations out of the same suspension are highly comparable, with about nine-tenths of the eggs appearing on the first cover from a tube. Also that dilution counts, and larval yields after culture from the same sample, insofar as has been tested, do not indicate but that the flotation counts are reflecting the egg content of the feces being examined.

3. A special problem arises as to the meaning of flotation examinations that show no eggs. As the two light positive cultures on specimens that were flotation negative show, small numbers of eggs may be present in the dung and be missed. An approximation can be made of the maximum size of such "missed" positives on the basis of the egg output per day of infected hosts.

In an earlier publication (Stoll, 1929) attention was drawn to a general relationship of ten times the weight in pounds of sheep representing the average amount in grams of pellet dung passed per day by the animal. Thus a 100 pound sheep on the average defecates 1000 grams of pellets in 24 hours. A "just positive" flotation count of 1 egg from a tube representing 1/2 gram of the dung specimen, would indicate a 2000 egg output in a day's time; a negative flotation, in the absence of other information about the animal, might indicate anything less than that, if only one flotation tube were examined from the specimen; similarly on lambs a just positive specimen might show as few as 200–300 eggs per day. This reaches down to the ova production of 1 or 2 normally egg producing females of the familiar strongyloid forms.

Henry and Morrison (1916) point out that while sheep pass about 23 pounds of dung per 1000 pounds live weight per day—a figure within 4 per cent of the original determinations of Stoll, 1929—in cattle this figure is 52 pounds. Not only does the bovine host pass 2¼ times as much feces per day as the sheep of equivalent live weight, but for equivalent ages has a live weight 5 to 10 times that of sheep. Thus the fecal output of individual cattle is 10 to 20 or more times that of sheep of similar age.

In figuring bovine fecal outputs per day it is conservative to use twenty times the weight of the animal in pounds as an estimation of the weight in grams of the daily defecation. Negative flotation examinations on cattle may mean that strongyloid eggs are being passed by an adult bovine host at levels of 10,000 to 20,000 eggs per day without being routinely demonstrated by one flotation tube. This would imply animals weighing 500 to 1000 pounds, "yearlings" or better. Additional flotation tubes examined from the same fecal sample would permit lowering the threshold value of the just missed case. This practice we have been following in more recent examinations.

Some further insight into the meaning of flotation results on cattle dung for strongyloid eggs is given in second examinations from the same

animal. Following examinations made in mid-winter when reinfection is absent, of 6 negatives on first fecal collection, later collections were similarly negative 2, 5, 6, 7, 7 and 8 weeks later; 2 other animals negative showed 2 and 6 eggs per gram 10 days later; a positive with 1 egg per gram was negative 2 weeks later; two positives of 20 and 27 eggs per gram showed 14 and 17 a week later. The indication, as in other types of observations, is that somewhat more reliability inheres in the positive data.

Milk feces of very young calves do not permit of satisfactory flotation examination. Also, there is insufficient evidence available to interpret flotation efficiency for *Capillaria* and *Trichuris* eggs in cattle dung. The indication is that *Moniezia* eggs are effectively floated.

OBSERVATIONS

The Field

Field VII (see map, Stoll, 1935), enclosed as a 16-acre pasture in 1931, occupies the top of a gentle slope about a quarter mile from the laboratory, and is not subject to drainage from any other fields. It was known to have had no grazing animals upon it, at least since 1922, and in 1928 and 1929 was in corn and rye. In 1930, preceding plowing for the pasture grass mixture in August, the only manure used was from a rabbit and guinea-pig colony which is in a separate building a mile from the main laboratory. The field, when fenced in 1931, was thus accepted as being free of helminth infestation for ruminants.

In 1933, following removal of the sheep from field VII, its area was greatly enlarged by adding 26 acres. This addition was likewise a field that for several years had been used only for cultivated crops, and having been in hay since 1930, was used for cattle pasturage without further seeding. These 26 acres similarly were not subject to drainage from other areas.

Care of the animals in field VII was by men working primarily in the small animal stock colony nearby. This eliminated regular traffic with the main laboratory and its environs, and aided the plan of keeping the new pasture insulated from extraneous infection.

The Cattle and Their Infections

On April 22, 1931, a Guernsey bull calf 5½ months old was taken to field VII. Nine heifers were added between May 1 and June 30. These ranged in age from 6½ to 10½ months, and were of Guernsey, Holstein and Jersey breed. While the animals had initially been bottle-fed, they had not been kept under known helminth-free conditions. Exposure to gastro-intestinal nematodes in the calf corrals appears to have been to light infestations of several species. Such infections were evi-

dently enhanced by pasturing 8 of the 10 calves at a nearby farm under Institute control. While no ruminants had been on this place for 2½ years, three of the calves were together for 6 months preceding transfer to field VII, and five others were with them or nearby during April and May before similar transfer. Two of the ten were not held on this farm, but one of these (not one of the two mentioned below) was known to be positive for *Trichuris ovis* when transferred to field VII.

Of interest in its bearing on the infections which developed in field VII is the further fact that the two oldest of the ten heifers, both born in June, 1930, were placed when a month old in paddock C (cf. Stoll, 1935). This contained sheep lightly infected with *Capillaria longipes*, *Nematodirus* sp., *Trichostrongylus colubriformis* and *Trichuris ovis*. These two calves were in paddock C from July 24, 1930, to April 2, 1931, then on the nearby farm until May 1, when placed in field VII.

No additional cattle were ever put in field VII, and, as mentioned, an animal once removed was not returned. Through breeding the herd was gradually increased, numbering 17 animals in January, 1933, 18 in March, 1934, 23 in December, 1935.

At the time the cattle were placed in field VII, fecal examinations were made, the one positive being an eight months' old heifer showing eggs of *Trichuris ovis*. As noted above, with nematode eggs demonstrable as effectively in bovine dung as in sheep, these cattle which were estimated to weigh 450 to 600 pounds could have been individually passing nine to twelve thousand strongyloid eggs per day although not detected as positives. Thus without having been unmasked by the initial examinations, infections in the cattle when they were placed in the new pasture may have been comparable in degree to those found in many of the sheep after a first season's contaminative grazing in field VII with the cattle, namely at the December, 1931, examinations.

The cattle were not examined again until January, 1933, which was 19–21 months and thus two grazing seasons after introduction to field VII. The original animals were now 26–31 months old and nine of them were lightly positive with strongyloid eggs, believed to be principally *Haemonchus*. The bull was negative. There were by then seven calves. Of these, six born April to July, 1932, were similarly lightly positive. A month-old calf was negative.

Fecal examinations were next made in March, 1934. The nine original animals of the herd were now 3½ to 4 years old, and with them were five 2-year-olds and four yearlings. Of the first nine, three were negative; of the other nine, one was not examined and one was negative. Eggs seen were of strongyloid type, although not alone of *Haemonchus* aspect, and all the infections as in 1933 were less than 50 eggs per gram, except for a 23 months old bull showing a count of 400 per gram.

In December, 1935, the cattle were again examined. Besides seven of the original herd, now 5 to 5½ years old, there were nine animals 1 to 4 years old, and seven calves 3 to 10 months old. All of the original and five of the nine younger adults were lightly positive, 1 to 16 per gram with strongyloid eggs. The seven calves had the following per gram counts: 110, 20, 100, 40, 30, 20, and 30. The youngest calf at age 6 weeks had milk feces in which no eggs could be demonstrated on repeated examination; at age 10 weeks a satisfactory examination was obtained, showing 30 eggs per gram as indicated.

Besides the above counts the last three calves, aged 3–4 months, also showed 2 to 4 *Nematodirus* eggs per gram, as did one of the oldest cows. One nine months old calf was positive for *T. ovis.*

In an effort at diagnosis of species, eggs from the 19 positives in December, 1935, were carefully studied and measured. The *Nematodirus* eggs, 179–205 μ × 95–107 μ, according to Tetley (1935) are beyond the usual range for *N. filicollis.* The total size range of the other strongyloid eggs was 51–93 μ long by 22–54 μ wide. No specific diagnostic value could be attached to these sizes, except that no eggs were small enough to classify for *Strongyloides* or large enough for *Chabertia*—a bit of evidence tending to confirm our failure to locate these two genera in field VII.

Supplementing the information from fecal examinations, are additional data from autopsies. On February 23, 1933, the original herd bull, then 27 months old weighing 1035 pounds, and negative to fecal examination in January, was killed, and in examination of the abomasum, 2 *H. contortus* were found. A bull calf 8 months old was killed the same day. His egg output in January had been 270,000 per day, and from his abomasum 29 *H. contortus* was recovered. A 6 months old bull calf, weighing 600 pounds, killed in October, 1934, showed no stomach worms. In these cases the gut was not examined except for tapeworms which were never encountered. In another bull calf 8½ months old, killed in December, 1934, stomach worms were likewise not found, but in examining the small intestine about 200 *Cooperia oncophora,* with small numbers of *Nematodirus* sp. and *Trichostrongylus* sp. were noted, together with 36 *Trichuris ovis* in the cecum.* The *C. oncophora* was parasitized by a *Giardia* species (Graham, 1935). In a cow of the original herd which died by accident in December, 1934, one *Setaria labiatopapillosa* was recovered from the peritoneal cavity.* No examination for gastro-intestinal worms was made of this animal. In another cow, 33 months old, killed in January, 1935, no worms were found in the small gut, but the abomasum was not examined.* In none of these animals was search made for *Gongylonema*; there were were no lung worms.

* Data from G. L. Graham.

The above autopsy information is fragmentary, partly because the helminths present in the herd had been only a minor interest in its maintenance and utilization, and no clinical verminoses had developed. In the extensive bovine gastro-enteric tract light infections, particularly with the smaller strongyloid forms and *Capillaria,* would not be readily demonstrable, especially in the presence of gross digestive debris in animals not fasted preliminary to slaughter. It cannot be said therefore that failure to find worms in the particular autopsy material examined meant freedom from helminths, although such parasites if present must have been in small numbers. Exceptional care, taken in the examination of a 9 months old heifer, killed in December, 1935, and in which 72 worms were found after rigid search illustrates this. There were no *Haemonchus,* the abomasum with six inches of duodenum attached showing only eight worms: 7 ♀ *Trichostrongylus axei* and 1 ♂ *Cooperia punctata.* In the small gut 59 worms were found: 1 ♂ *T. axei,* 6 ♂+7 ♀ *Nematodirus helvetianus,* 3 ♂+3 ♀ *Cooperia curticei,* 1 ♂ *C. oncophora,* 19 ♂+18 ♀ *C. punctata,* and 1 young ♀ *Trichuris ovis.* There were no worms in the large gut, but the cecum yielded 1 young ♂+2 adult ♀ *Trichuris ovis.* In the esophagus were 1 ♂+1 ♀ *Gongylonema scutatum.* This animal was marked not only by a small worm count, but practically no young nematodes.

A young bull 6½ months old, still suckling and weighing 450 pounds, was removed from the pasture on March 26, 1936, and killed 4 days later. Again exceptional care was taken in the worm count and 2013 nematodes recovered: from the abomasum only 2 ♂+9 ♀ *T. axei*; from the small gut 1 ♂+2 ♀ *T. axei,* 3 ♂+1 ♀ *Cooperia curticei,* 713 ♂+910 ♀ *C. oncophora* (of which 590 were young worms), 18 ♂ *C. punctata,* 71 ♂+119 ♀ *N. helvetianus* (with 108 additional young forms), and 2 ♂+3 ♀ *Capillaria longipes*; from the cecum 3 ♂+48 ♀ *Trichuris ovis* (of which 3 were juvenile). The peritoneum, lungs, rumen, esophagus and large gut were negative. The large numbers of undeveloped worms, representing 36 per cent of both the *Cooperia* and *Nematodirus* indicated active reinfection during an unseasonably early spring of an animal whose egg count in December at 3 months of age had been only 50,000 per day. Failure to find *Haemonchus* and *T. colubriformis,* known present in the pasture in 1933, as well as the positive evidence of *C. longipes,* brings the field VII record to date.

The Sheep and the Infections They Contracted

There were placed in field VII on April 22 and on June 23, 1931, fourteen bottle-raised lambs, then 8–17 weeks old. These had each been examined by flotation the week before transfer and gave no evidence of helminths. In the period 1925–31 no case of helminthic infection had

been demonstrated in over 60 lambs reared under the conditions found successful by Smith and Ring (1927). In July twin kids, likewise bottle-raised, and 7 weeks old, with two more lambs, respectively 6 and 9 weeks old, were taken to the pasture. Fecal specimens from these four animals collected July 13, the day before transfer, were negative in the case of the young goats, but the two lambs showed 2 per gram of eggs identified as of plant nema origin. These lambs, but not the kids, had been fed fresh grass for 2 weeks. While the finding of plant nema eggs is not unusual in such circumstances, in order to guard against possible error the two lambs were removed from the field after having been there 2 days. Subsequent examinations following change of feed showed no further signs of the plant nema eggs, but the lambs were not returned to field VII. Two of the first batch of ten lambs introduced were brought to the laboratory May 5, and in eight fecal examinations each, in the next 7 weeks, gave no indication of having contracted infection in their 13 days in field VII. These lambs, incidentally, constitute a fair control that the pasture was uninfested when opened.

No further sheep were placed in the field, the remaining two rams and ten ewes being held for breeding. The surplus male was removed in December and the two young goats in February, 1932. The flock numbered nineteen sheep and lambs in the 1932 season, and in the spring of 1933 before all were removed from field VII there were nine 2-year-olds, seven yearlings and nineteen lambs.

On July 14, 1931, after the eight remaining lambs of the April group had been in field VII 12 weeks and four of the June group had been there 3 weeks, fecal examinations showed four of the former and one of the latter lightly infected, 1 to 4 per gram of strongyloid eggs. At the next examination in December, when lambs had been pastured with the cattle for 20 additional weeks, all without exception were lightly positive, 2 to 60 per gram, with eggs most of which in size range fit *Cooperia*. Positives of the eggs per day level found in the lambs in July and in half of them in December would not have been detected in cattle by our examinations.

One of the lambs was brought indoors at this time for additional examinations. In a period of 3 months, during which time it was not exposed to further helminthic infection, it showed in four of twelve examinations 2 to 4 per gram of strongyloid eggs. On one occasion *Capillaria* eggs were noted. At autopsy of this ten months old animal February 29, 1932, 2 ♂ + 1 ♀ *Trichostrongylus colubriformis* alone were found in the small gut, the *Capillaria* having evidently been lost before death. The lungs, esophagus, stomach and large gut were negative.

The two young goats were brought to the laboratory in February. They both showed light infection, like the lamb, with strongyloid para-

sites, and one also with *Capillaria,* contracted in field VII. Their later experimental use precluded determination of the contaminative species.

The sheep were next examined in May, 1932. Nine spring lambs, 1 to 6 weeks in field VII, were all negative except for coccidia; of the eleven yearlings, seven were positive for strongyloid eggs, 2 to 6 per gram, and two for *Capillaria.*

In October, 1932, after five more months on pasture, the sheep were re-examined. On this occasion, not only were all without exception positive, but the egg counts were higher. In the yearlings they ranged from 10 to 800 per gram, in the lambs from 900 to 3300 per gram.

At this time two lambs each 5 months old, which had been born in field VII, were brought to the laboratory and maintained in clean stalls. Each showed about 2500 eggs per gram. One of them was held for 17 days and slaughtered, a preliminary 48-hour fecal collection showing 1,500,000 egg output daily. At autopsy there were recovered 267 *H. contortus* from the abomasum, 32 *T. colubriformis* and 3 *C. curticei* from the small gut, with 2 *T. ovis* from the cecum. In the 4th stomach also were the characteristic lesions of *T. axei,* although the worms were not observed. There were no large gut worms, and the esophagus and lungs were negative. The worms of each species found were all mature, the females egg-bearing.

On examinations in the spring of 1933, most of them in March, the nine remaining 2-year olds, and seven remaining yearlings, showed eggs of the strongyloid type in greatly decreased numbers compared to the preceding October. Of the nineteen suckling lambs removed from field VII at 6 to 19 weeks of age, at least 15 had become infected. One 2-year old ewe and one spring lamb at autopsy June 2, too soon after departure from field VII to have contracted infection elsewhere, each had mature *Nematodirus* sp. and *Capillaria longipes.* Under similar conditions two other 2-year old ewes showed respectively 4 and 11 specimens of *Gongylonema scutatum* in the esophagus.

Worm Species Involved

A synopsis of the eight genera and eleven nematode species found in field VII animals is given in Table I. Although secondarily contracted by the sheep from the cattle, the seven genera and eight species recovered from the ovine host are common to it in the United States. *Nematodirus helvetianus,* determined from two bovine hosts in field VII, is only presumptively ascribed to the sheep, the worms secured from a lamb and ewe in June, 1933, not permitting definitive diagnosis. *H. contortus* of cattle strain has been shown by Ross (1931) to go readily into lambs. (Ross found a better percentage of larvae developed than in the case of a calf infected with the same strain, although this calf may have been

already infected, as a control calf showed more *Haemonchus* than did the experimental infection.) In view of the fact that *Haemonchus* has not been recovered from any field VII animal at autopsy since 1933, there is a possibility that the twisted wireworm infestation there is dying out. If so, it becomes an interesting question as to whether it is causally related to the absence of sheep in the pasture.

Certain of the species found in the cattle are likewise of interest. *Setaria labiato-papillosa,* noted in routine autopsy of one cow from field VII, has been recovered on two other occasions* from cattle at this laboratory. *Cooperia curticei* and *Trichostrongylus colubriformis* are both usually considered sheep forms, although the latter is also known from other ruminant hosts. Both have also been previously noted in cattle, the former by Cameron (1923) in Scotland, the latter by Mönnig (1923) in South Africa. *T. colubriformis* has not yet been recovered from field VII cattle at autopsy, but was found at autopsy of two lambs which were infected in field VII in 1931 and 1932 respectively. A similar situation existed for *Capillaria longipes,* until March, 1936. It was present in three sheep and a goat in 1932 on fecal examination, and recovered at autopsy in 1933 from two sheep, including one that had been positive by flotation. While this secondary infection of susceptible sheep can be looked upon as a delicate test of the presence of a particular species in the cattle, and while this is especially true of *Capillaria,* inasmuch as fecal examination for the egg and autopsy examination of the bovine gut contents and wall for the capillarids themselves offer peculiar difficulties to the demonstration of this genus, it was a satisfaction to encounter 2 ♂ + 3 ♀ *C. longipes* from the field VII bull calf autopsied in March, 1936. This appears to be the first demonstration of this parasitic species in cattle, although Dikmans (1930) diagnosed the closely related *C. brevipes* from a Virginia calf. In a recent paper Freitas and Lent (1936) agree with Petrow and Orlow (1930) that on morphological grounds *Capillaria bovis* Schnyder 1906 is identical with *C. longipes* Ransom 1911, and that *C. bovis* thus becomes the acceptable name for the form in both sheep and cattle. Our observations would appear to support this contention on both morphological and physiological grounds.

Reference has been made to the fact that two of the calves before transfer to field VII had been 8 months in paddock C where over a period of three successive years a light infestation of *Trichostrongylus colubriformis, Nematodirus* sp., *Capillaria longipes* and *Trichuris ovis* had become established, as demonstrated by autopsies. It is possible *Cooperia oncophora* was also in this paddock, but the evidence is inconclusive. It is improbable that any other species was present. (It may be emphasized

* By Theobald Smith; identification by J. E. Stumberg.

TABLE 1.—*Nematodes demonstrated in animals from a new pasture, field VII, having been established there by infected cattle*

	In cattle	In sheep
Haemonchus contortus	+	+
Trichostrongylus axei	+	+[1]
" *colubriformis*	(+)[2]	
Cooperia curticei	+	+
" *oncophora*	+	
" *punctata*	+	
Capillaria longipes	+	+
Nematodirus helvetianus	+	(+)[3]
Trichuris ovis	+	+
Gongylonema scutatum	+	+
Setaria labiato-papillosa	+	

[1] Lesions in abomasum; worms not recovered.
[2] Ascribed to cattle because of presence in associated sheep.
[3] *Nematodirus* recovered, species uncertain, but in size range appropriate for *N. helvetianus*.

again that the lambs involved in field VII had not been in this paddock, nor elsewhere out-of-doors, until transfer to field VII.) It is known that one calf, not in this paddock, was infected with *T. ovis* when transferred to field VII, so that the whipworm cannot have arisen from paddock C exclusively. The *C. longipes* and *T. colubriformis* infections may have come from the two animals that had been in paddock C. If so the demonstration is doubly of interest, as having gone from sheep to cattle to sheep.

There is some evidence that the two calves may have introduced *Gongylonema* to paddock C. No infections with this species were known in paddock C up to 1931, on the basis of autopsies on 4 sheep, although it was known to be established in our permanent pasture, field I. Following the 8 months in which the calves were in paddock C, and despite subsequent plowing and reseeding, *Gongylonema* infections have appeared in sheep grazed there, 8 or 16 autopsies appropriate for this determination in 1933–34 being positive. While this is presumptive evidence that the calves brought *Gongylonema* to paddock C, as well as to field VII, it is to be recalled that this parasite may be transmitted to susceptible ruminants by migration from pasture to pasture of the appropriate dung beetles involved as intermediate hosts. Ransom and Hall (1915) and Baylis, Sheather and Andrews (1926) were able to infect sheep with *Gongylonema scutatum* which developed in insects that had been fed eggs of bovine origin.

In considering the helminths involved in field VII it is of interest to note that several which are common in our permanent sheep pasture and in paddock A (cf. Stoll 1935) failed to appear in the natural experiment described. These include the genera *Ostertagia, Bunostomum, Strongyloides, Oesophagostomum, Chabertia* and *Dictyocaulus* among the nematodes (and two *Moniezia* species). There has been no hint that any of these has been present in field VII during the five years 1931–35. This indicates the degree of insulation that was fostered and is thus demon-

strated between field VII and other sources of ruminant helminths here. Cattle species of two of these genera, namely *Ostertagia ostertagi* and *Bunostomum phlebotomum,* we have found in bovine hosts from local sources after autopsy at this laboratory. On the other hand, nodular and lung worms seem to be rare in cattle in this part of New Jersey, judged by their absence in autopsies of cattle here.

Students of the biology of the nematodes mentioned will be especially impressed with the complete absence of *Strongyloides,* which here, as in other places in the world where sheep are studied, is a contaminant established with marked ease. Its absence would seem to be a good indication too that feral mammals, especially rabbits, were not responsible, at least during the period 1931–33 when sheep were present, for cross transfers between field VII and fields I–II, the permanent infestive pasture a half-mile away, nor between field VII and infested paddock A at half that distance (Stoll, 1935).

Besides the nematodes, the fluke, *Cotylophoron cotylophorum,* of the rumen (the only ruminant fluke thus far recovered at Princeton), and *Moniezia* found at autopsies here respectively in 1929 and 1930, have not been in field VII. The absence of the cestode is determined not only from its non-appearance in any examination, fecal or postmortem, but by the fact that the sheep in field VII were shown susceptible to infection when introduced into our tapeworm infested pasture in 1933. *Moniezia* is present in cattle of the region, having been recovered from a yearling heifer slaughtered in March, 1930. This animal also harbored about 4500 nematodes, including *H. contortus, O. ostertagi, B. phlebotomum, Nematodirus* sp., *Trichostrongylus* sp., *Cooperia punitata,* and 4300 *C. oncophora.* It came from a herd believed to have had two recent fatal cases of verminous gastro-enteritis.

TABLE 2.—*Nematodes of cattle found at Princeton, with comparative American listings. The species involved in field VII are noted with an asterisk**

	Ransom 1911 U.S.A.	Price 1928 Texas	Swales 1933 a, b Canada	Stoll this article Princeton
Trichuris ovis	+	+	+	*
Capillaria longipes				*
Oesophagostomum radiatum	+	+	+	
Chabertia ovina	+			
Bunostomum trigonocephalum			+	
" *phlebotomum*	+	+	+	+
Trichostrongylus axei	+	+		*
" *colubriformis*				*[1]
Haemonchus contortus	+	+	+	*
Ostertagia ostertagi	+	+	+	+
Cooperia curticei				*
" *oncophora*	+			*
" *pectinata*	+			
" *punctata*	+	+	+	*
Nematodirus filicollis	+			
" *helvetianus*				*
Dictyocaulus viviparus		+	+	
Ascaris vitulorum	+			
Gongylonema scutatum	+	+	+	*
Setaria labiato-papillosa		+	+	*

[1] See Table 1.

High *Moniezia* incidence is not, however, reported in the literature for cattle. In Wales, Morgan (1924), Jones (1926) and Lewis (1930) indicate a total of 9 positives in 89 cattle examined after slaughter, Jones remarking also the butcher's experience that tapeworms are of rare occurrence in cattle. These authors, and others, have indicated, however, a high incidence of *Moniezia* in sheep in Wales, as is true here. Schnyder (1906) found 2 in 17 cattle with *Moniezia* in Switzerland and believed the infection to be more common there than generally supposed.

A synopsis of the nematodes of cattle thus far found at Princeton, in comparison with the findings of Ransom (1911) for the United States (alimentary tract species only), Price (1928) for Texas cattle, and Swales (1933 a and b) for Canada, is given in table 2.

Egg Outputs of Infected Hosts

Emphasis has been placed on the *fact* of infection of the sheep by the worms brought to field VII in the cattle. Available evidence as to the *degree* of infection of both hosts is of interest in that while there are several genera and species of nematodes involved in field VII, the number and variety of these species is considerably less than in our permanent, infestive pasture, where sheep have usually developed either serviceable immunities against these worm species or have died with the accompaniment of large infections (unpublished data).

Not only have there been no noticeable clinical effects due to verminosis during the period under observation, but both cattle and sheep have been marked for their excellent condition, early breeding, and general evidence of well-being. Calves, which weigh from 40 pounds in primiparous Jerseys to over twice that in multiparous Guernseys, suckle their dams *ad libitum,* and gain weight rapidly. Thus a bull calf, half Guernsey and half Jersey, born in April 1934 was still a suckler when killed at over 6 months of age, with a live weight of 600 pounds, and a Guernsey heifer calf born in March 1934 was still suckling when killed at 9 months of age. The lambs in 1932 and 1933 were also reared on their dams.

A comparison of the number of worms in the hosts without regard to species is revealed to some extent indirectly in the daily egg outputs obtained by multiplying the egg counts per gram by the estimated fecal bulk of the hosts, as earlier explained. In the examinations of January 1933, nine of ten of the original herd were positive. On the basis of estimated individual weights of these nine animals and their feces, the daily egg outputs averaged 130,000 (range 40,000–430,000). This was after their second grazing season in field VII. At the same time the six positive calves 7 to 9 months old (an additional one month old calf was negative) at the end of their first grazing season, showed daily 170,000

eggs (range 50,000 to 290,000). In examinations in March 1934 after another grazing season, the six positives of the original herd averaged 170,000 eggs per day (range 40,000–320,000); six of the other seven younger positives, 150,000 (range 60,000–500,000). The seventh positive, a 2-year old breeding bull of Guernsey blood, had gone from 100,000 eggs per day in January 1933 to 7 million. At the next examination 21 months later in December 1935 this animal's count was down to 1 egg per gram, estimated at about 20,000 per day, a reduction in the interim of approximately 99.7 per cent. At this time 4 of 16 animals 16–56 months of age were negative; the 12 positives showed an average of 80,000 eggs per day (range 20,000–290,000). In contrast, seven calves had an average of 400,000 eggs per day (range 50,000–1,170,000). In this group of calves, the three youngest, born in August to October, averaged 70,000 eggs per day; the other four were 8 to 10 months of age and reflected more exposure to infection through comparatively more grazing in an average ten times higher at 700,000 eggs per day.

The above evidence of greater susceptibility to and acquisition of, gastro-intestinal nematode infection in the younger bovine hosts, is supported by an isolated observation on a herd of 17 cattle, mostly Jerseys, which grazed on extensive pasture in Mississippi. Six of these animals were given fecal examinations in mid-winter. Four cows, 3 to 5 years old, which had never had anthelmintics, were negative. A yearling heifer and yearling bull, despite dosing with copper sulphate 3 weeks before this examination, were contrariwise, positive, with strongyloid egg outputs estimated at 800,000 and 1,500,000 per day. Presumably these were much higher before treatment, and yet the total amount of exposure to infestation for these younger animals was obviously only a fraction of that of the older cows which had had from 2 to 4 more years grazing.

The fact of greater insusceptibility of older bovine hosts to nematode parasitization is frequently hinted in the literature, and Daubney (1929) states: "It is the exception to find adult cattle heavily infested . . . and for practical purposes adult healthy cattle may be regarded as highly resistant to these infections. Calves on the other hand, while not displaying the high susceptibility of sheep, are less resistant and may harbor heavy infestations, both of wireworm and *Trichostrongylus* species, with resultant gross infestation of pastures."

Returning to the situation in our field VII, the increase in pasture infestation in the fifth grazing season, 1935, over that in the second grazing season, 1932, seems distinct, from the comparison of 170,000 average eggs per day in 6 calves 7–9 months old examined in January 1933, and 700,000 average eggs per day in 4 calves 8–10 months old examined in December 1935. It is possibly greater than the figures indicate

inasmuch as the 1932 infections evidently involved *Haemonchus* which is a more fecund form than the *C. oncophora* that appear prominently in recent autopsies, with the concomitant absence of *Haemonchus*. This definite increase was likely retarded by the opening of the field VII addition of 26 acres in the summer of 1933, for calves grazing their first season in 1932 had access to 16 acres, those grazing their first season in 1935 had access to 42 acres. Comparative stocking was 1/2 cow per acre in 1935, 1 cow plus 1 sheep per acre in 1932.

The 1932 season permitted a direct comparison of the amount of infection contracted by lambs and sheep exposed to the same infestation. Reference has been made to the fact that in May 1932, of eleven yearlings and nine spring, suckling lambs, all of the latter and four of the former were negative on fecal examination, the seven positive adults showing light infections of 2000 to 8000 eggs per day, a grade that would have gone undetected in the cattle. In October, after 5 months' grazing season, all were positive. The egg outputs in ten adults (one had been withdrawn) averaged 400,000 per day (range 10,000–1,000,000) ; in nine lambs 1,900,000 (range 800,000–3,000,000). It is probable that October was too late to catch the peak counts in these animals, and the evidence does not distinguish whether there is indicated a gradually developing resistance in the adult sheep due to the fact that they had been carrying low grade infections for many months (all had been shown lightly positive at the preceding December examinations), or whether so-called age resistance, as such, was influential. We have been able to demonstrate (unpublished data) immunization to *H. contortus* under indoor conditions by long drawn-out, low grade infections. The interest that attaches here is that whichever explanation is appropriate, it applies against infection with cattle strain worms in the sheep. Reference has been made to the fact that a lamb from the group taken indoors following these October examinations, showed an egg output of 1,500,000 per day, and at autopsy 302 strongyloid worms.

At examination in March 1933, ten 2-year old sheep showed 80,000 eggs per day (range 10,000–200,000), and eight yearlings 50,000 (range 10,000–150,000), notable reductions from the higher counts of October 1932. Of eleven of these yearlings and 2-year olds, transferred during the spring of 1933 to field I–II, which was permanent infestive pasture with mixed ovine nematodes of the region, none showed striking egg counts there, except for one nematode species. This was *Strongyloides papillosus*. High *Strongyloides* counts in older animals have been unfamiliar unless such animals have previously been uninfected, which gives further evidence of the absence of this genus in field VII.

No examinations of the cattle were made in October 1932, when the sheep and lambs had their highest counts, so that direct comparison

between the two host species at that time is lacking. Insofar as cattle examinations of January 1933 are comparable with the next sheep examination in March 1933, the net worm burdens as measured by eggs per day, were not of greatly differing magnitudes. Thus nine adult cattle in January averaged 130,000 eggs per day, 10 adult sheep in March 80,000. Also 6 bovine yearlings born April–July 1932, in January 1933 averaged 170,000 eggs per day, 8 ovine yearlings born March–May 1932, in March 1933 averaged 50,000.

DISCUSSION

While the total number of species of nematodes found in cattle over the globe is impressive, there are, as mentioned earlier, relatively few data as to the status of bovine nematode infections. Hall (1930) in his "parasitological résumé," discusses nematodes as a first topic under parasites of sheep but limits discussion of parasites of cattle to anaplasmosis and liver fluke.

Forty years ago M'Fadyean (1896) published on the occurrence of "verminous gastro-enteritis in cattle" in England, and summarized earlier reports. Evidently *Trichostrongylus axei* was prominently involved. A serious outbreak of mixed verminous bronchitis, gastritis and enteritis in cattle, considered to have caused the loss of 10,000 head in the two wet years 1899–1900 in Texas, is described by Stiles (1901). Tunnicliff (1932) found among 500 range calves being wintered in a hay meadow in Montana, 15 became sick and two died. Besides coccidiosis, these had numerous *C. oncophora* and *N. helvetianus*. On a change of feed, all sick animals promptly recovered, whether or not drenched with copper sulphate solution. Baker (1933) in an article on *T. axei* noted a heavy infection in a 9 months old calf suffering from chronic bloody diarrhea, no coccidia being found. Taylor (1934), describing methods of counting worms in dead animals, recorded as his one bovine case 140,000 *Trichostrongylus* sp. in the fourth stomach of a 2-year old heifer "from a naturally occurring outbreak of parasitic gastritis," presumably in an English herd. The same author (1935) finds egg counts of "only 400 to 600 e. p. g. are frequently seen in cases of parasitic gastritis."

Boynton and Wharton (1916) observed extensive losses in a herd of 90 cattle in the Philippines, ascribed primarily to heavy mixed infections of *Haemonchus, Bunostomum* and *Oesophagostomum,* and secondarily to typhoon exposure. Montgomery (1916) reported for British East Africa considerable mortality among cattle which were shown postmortem to contain a large number of worms in the fourth stomach, and Daubney (1929) in Kenya found outbreaks with *Haemonchus* "do occur among calves, in which enormous infestations are seen." LeRoux (1930) mentions that the familiar strongyloid forms "have definitely

been found causing serious mortality on the Natal Coast and Zululand, where helminthiasis has undoubtedly been at times mistaken for nagana." From one upland stock farm in northern Natal, however, Ortlepp (1926) had evidence only of *Ascaris vitulorum* (and *Moniezia*) in the dung of two calves following vermifuge, although sheep on the same farm had many nematodes of several species. In abattoir studies in Wales, Morgan (1924) noted that "on the whole the animals examined [25 cattle] proved to be remarkably free from worms"; Jones (1926) found 1 *Moniezia* as the only parasite in 16 cattle although he endeavored to select those in comparatively poor condition; and Lewis (1930) reported the helminthic parasites collected from 48 cattle to be "very few." Obviously many factors are at work to produce these net results. Occasionally, as Hall (1927) concluded in connection with his failure to find any gastro-intestinal nematodes whatever in cattle in Central America, abundant range and alternating wet and dry seasons may be presumed to eliminate them completely.

This relatively sparse literature upon the gastro-intestinal nematodes of cattle may imply trouble with them only occasionally, for Schnyder (1906) had already stressed the problem three decades ago. He found that Swiss cattle were considerably parasitized, species by frequency of occurrence being "*Strongylus ostertagi, S. retortaeformis, S. curticei, S. oncophorus, S. filicollis,* and *S. contortus.*" He believed that a not uncommon, chronic, afebrile diarrhoea, locally termed "Kaltbrändigkeit," was causally related to heavy infections, and that a few thousand strongyloid worms could provoke symptoms.

Observations of herds of cattle in relation to their changing worm infections on pasture have not been found in the literature. The herd that has been under observation here has the advantage from the standpoint of the parasitological findings, that it has been a "closed universe" since its initiation in a clean pasture. Except for the first cattle established in field VII, no additions have been made to it except by breeding in the area, and absence of contamination of the area by extraneous infection has been, from the helminthological point of view, ideal. Moreover, the herd is a grazing herd par excellence, its use being not for dairying but to supply animals to the laboratory. These are obviously somewhat special conditions as compared to the way cattle are ordinarily handled by the farmer. They are, on the other hand, natural conditions in the true sense of the word for observation of what occurs when *Bos taurus* is subsisting primarily on permanent pasture which was originally helminth-free and has been gradually developing the grade of infestation permitted by local environmental and climatic conditions, with stocking at the rate of 1/2 to 1 animal per acre. The 42 acres of pasture involved have not been reseeded since 1930.

The fact that sheep are readily infected by helminths of cattle strain, when in association on pasture with infected cattle, is not unlooked for, and is here in direct demonstration. The observation of Tetley (1934) that lambs grazed "on pasture previously occupied only by cattle" contracted *O. ostertagi* and *C. punctata,* two species regarded primarily as cattle forms, is similar evidence. Tetley also recovered from sheep the species *N. helvetianus,* heretofore believed an exclusive bovine parasite.

Tetley is led to conclude that "cattle are the chief reservoir hosts of nematodes of sheep in Manawatu district. . . . Frequently dairying is combined with sheep farming, so that they become important sources of infection." This experience in New Zealand tallies with an observation from South Africa by Mönnig (1931) that "cattle seem to have a very bad influence on the worm infestation of sheep, where these animals run on the same pasture." One interpretation of "bad influence" is given by these Princeton observations of just such an association.

On the other hand, Ross (1931) in Australia, after finding that calves failed to become as heavily parasitized as lambs when both were experimentally infected with *Haemonchus* larvae of *sheep* origin, and noting that "cattle over one year old are very rarely heavily parasitized with *H. contortus,*" concludes "it is possible to advocate the rotation of sheep and cattle in order to reduce the risk of infestation of the former." Daubney (1929) writes in substantially similar vein. But Hall (1917) in suggesting the alternation of ruminants and non-ruminants on pasture, feels "the fact that all the ruminants are infested with the deadly stomach worm makes it undesirable to rotate cattle, sheep and goats with one another." In emphasizing that "permanent pastures perpetuate parasites" as "such pastures are dangerous—for that kind of stock," Hall suggests instead alternation of ruminants and non-ruminants on the same pasture. Thus "ruminants may follow horses," but to the extent that *Trichostrongylus axei* constitutes a troublesome verminosis of cattle or other ruminants, even such advice may require revision, inasmuch as Baker (1933) has shown this ubiquitous species to occur in horses in New York State.

Regarding the cognate problem, of the effect on cattle of increased pasture infestation produced by sheep, field VII has an unique interest, in that the worms established there are of "cattle strain." This presence of the sheep with the cattle during the first two grazing seasons did not increase the infestation of the pasture markedly from the standpoint of the observed cattle infection levels. That this meant reduced infectivity of the nematodes for cattle following sheep passage seems doubtful. It is more apt to be related to the comparative goodness of nematode larval habitats (culture conditions) represented by cattle and sheep dung, and the comparative grazing habits of each host in relation to dung deposits.

It is worth holding in mind that to the extent lambs and calves, or sheep and cattle on the same pasture show equivalent egg outputs from their nematode infections and thus inferentially similar infections, such similarity has in it also the suggestion that cattle may be many times as resistant as sheep to the given pasture infestation. Such a conclusion implies that food intake is in similar ratio to the dry fecal bulk of these two hosts, that they crop similarly infested herbage and thus ingest similar numbers and kinds of infective nematodes in ratio with the amount of forage eaten, and that such nematodes at sexual maturity shed about the same number of eggs in each host species. While it is unlikely that these factors would always go along together, we have no convincing information to indicate that just such an average effect may not be true. Certainly the disparity in food intake by cattle and sheep on pasture is such as to favor extensive verminoses in the former. "On pastures suitable for either cattle or sheep, 5 ewes may be considered the equivalent of 1 cow" (Semple, Vinall, Enlow and Woodward, 1934). Henry and Morrison (1916) tabulate similar dry weight food requirements for both cattle and sheep per thousand pounds live weight. Moisture determinations on the characteristic type of feces passed by sheep and cattle on pasture confirm these feeding figures and indicate that the same amount, between 8 and 9 pounds, of dry weight of dung is passed daily by both cattle and sheep per thousand pounds live weight. Without expressing as a numerical ratio the greater insusceptibility of bovine as against ovine hosts with similar egg outputs on the same pasture with mixed infestations, that such a factor is demonstrable in favor of *Bos taurus* seems definite.

The question arises as to whether the data thus far obtained from the cattle in field VII give a true reflection of the grade of bovine parasitisms occurring there. In one way they do not. Because the fecal examinations have been made in what is essentially the non-reinfection period of the year, the true height to which the parasitic levels have risen during the grazing period is obscured, as the sheep data indicate. As far as the net effects on the hosts are concerned, however, the conclusion is clear not only that there have been no sustained helminthiases of disturbing size in either the cattle or sheep associated with them, but that infection levels are by mid-winter reduced to where, judged by our sheep experience, they would be accounted essentially unimportant pathologically.

It remains for the future to show whether five years' observation "from scratch" of field VII gives an adequate picture of the reinfection problem there from the standpoint of the cattle, even based on mid-winter examinations. It is conceivable that field VII through cumulative effects or oscillation of climatic factors may in future permit the development of verminous infections of a grade thus far absent. If so, the particular

nematode species involved will be of interest, for there is evidence from the autopsy data that *T. colubriformis* did not establish well in the cattle, that *H. contortus* is dying out in field VII, and that *C. oncophora* infections are increasing. The observed fall and rise of the two latter species may reflect not alone differential susceptibility of the bovine host to them but the poor overwintering ability of the twisted wireworm larvae on pasture compared to the marked survival of *C. oncophora* under the same conditions. It is also predictable, by analogy with our findings on sheep, that *Capillaria longipes* and *Trichuris ovis,* once they infest a field, will maintain themselves persistently, by reason of the resistance of the egg stage on pasture.

SUMMARY

In 1931 there was established a new pasture which, by location and previous history, was free of helminthic infestation. To this area ten lightly infected calves brought a variety of nematodes, eleven species of the eight genera *Haemonchus, Trichostrongylus, Cooperia, Nematodirus, Capillaria, Trichuris, Gongylonema* and *Setaria* having been demonstrated. Eight species were secondarily transmitted to a group of lambs reared free from helminths which, with their later offspring, were grazed with the cattle until 1933.

The pasture has been maintained as a "closed universe" of infection, no new animals being added except by breeding within the area. Clinical verminoses have been absent throughout a five-year period. Differences in infection levels, however, are demonstrable between adult cattle and calves in terms of strongyloid egg outputs per day. Besides this greater insusceptibility of older animals, increased pasture infestation is demonstrable in 1935, the fifth grazing season, as compared to 1932, the second grazing season, although stocking in these two years was ½ head in 1935 compared to 1 head per acre in 1932.

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RESEARCH NOTES

A COLONY OF TRICHOMONAS-FREE RATS

Everyone who has done experimental work with parasitic Protozoa knows how important it is to obtain parasite-free laboratory animals. The rat is one of the best hosts for experimental work and trichomonad flagellates are among the most favorable Protozoa with which to work. Unfortunately a high percentage of the rats in the colony we have maintained in our department for the past 18 years are infected with trichomonads and often with other types of intestinal Protozoa. Several years ago we found that we could eliminate trichomonads from rats by treatment with carbarsone (Hegner and Eskridge, 1935, Amer. Jour. Hyg., 21: 135–150). Last year we started a colony with 6 rats, 4 females and 2 males, which had been treated with carbarsone and which we have maintained to date (May 6, 1936) free from trichomonad flagellates.

Six albino rats 6 weeks old were given one dose of carbarsone intragastrically on November 13, 1935. The dosage was one gram of carbarsone per kilo of body weight in a 5 per cent solution and suspension in 2 per cent sodium bicarbonate. Each rat received approximately 5 cc. of this mixture. At the time the drug was administered the rats were all found to be positive for trichomonads. *Giardia, Endamoeba,* and *Hexamita* were also commonly present in our colony. After treatment the 6 rats were kept together in one cage on a wire screen through which their fecal pellets dropped into a pan beneath. The rats were bred regularly from December, 1935, to May, 1936. The pregnant mothers were isolated and in course of time several cages were necessary to care for the progeny properly.

Once per week all of the rats in this colony were examined by the fecal-pellet-culture method that we devised a year ago (Hegner and Eskridge, 1935, Amer. Jour. Hyg., 22: 307–321); the cultures were never positive for trichomonads. On January 21, 1936, the colony was removed to another part of the building so as to minimize the possibility of reinfection due to accidental contamination. On March 3, 1936, 2 young 5 weeks old were killed and examined; *Endamoeba* were present, but no trichomonads. On March 9, 1936, one young 6 weeks old rat was killed and found to be negative for all intestinal Protozoa. At this time 10 of the offspring of the original 6 rats were removed and used in an experiment on the effects of allantoin on *Trichomonas hominis* in rats. On April 8, 1936, 8 more young were used for an experimental study of immunity of rats to infection with *Trichomonas hominis.* On May 5 and 6, 1936, 4 of the original rats and 13 young were killed, the contents of the intestine carefully examined by the smear method and three culture tubes containing 10 cc. of Ringer's solution and 0.5 per cent Loeffler's dehydrated blood serum were inoculated with cecal contents from each rat. At the same time 17 rats from our regular colony were similarly examined and cultures of cecal material made.

The results of these examinations are as follows:

The 17 trichomonas-free rats were all negative for trichomonads by smear and culture. Thirteen of them were positive for *Endamoeba*; and 3 of the 4 rats (2 males and 2 females) with which the colony was started were positive for *Giardia.* No *Hexamita* or other types of intestinal Protozoa were found.

The 17 control rats were all positive for trichomonads both by smear and by culture and all but one of them contained one or more of the following types of intestinal Protozoa; *Giardia, Hexamita, Endamoeba.* Four control rats harbored 4 types each, 9 rats contained 3 types, and 3 rats 2 types.

This colony, therefore, which was begun with 6 rats increased during the period from November 13, 1935, to May 6, 1936, to 50. All of these, except 7 young one week old, were examined at least half a dozen times by the fecal-pellet-

culture method and found to be free from trichomonads; 2 were killed on March 3, one on March 9 and 17 on May 5 or 6, and all found to be trichomonad-free by smear and culture. Two of the original 6, 3 young 6 weeks old and 7 young one week old have been retained with which to continue the colony. In course of time we hope to eliminate *Giardia* and *Endamoeba* also thus securing a colony free from all intestinal Protozoa.—Robert Hegner and Lydia Eskridge, *School of Hygiene and Public Health, Johns Hopkins University.*

SOME NOTES ON CESTODES FROM NEBRASKA

Recently a collection of worms from a "bull" dog yielded 120 fully developed normal taenioid tapeworms, 4 triradiate and one tetraradiate specimen. The polyradiate worms and, as far as determined, all the normal worms belonged to *Taenia pisiformis* Bloch. There were also 12 entire *D. caninum.* The specimens mentioned here were collected by Mr. H. F. Michels.

A search of the literature shows numerous cases of triradiate forms but only three previous records of tetraradiate tapeworms, two of which, are from the dog. Foster (1916, Jour. Parasit., 2: 7–19) reported on a single segment of *T. saginata.* Cram (1927, Jour. Parasit., 14: 54–55) has described a specimen of *T. balaniceps* taken from a dog at Denver, Colorado; and the Dobrovolnys (1935, Trans. Am. Mic. Soc., 54: 22–27) a specimen of *T. pisiformis* from a dog at Manhattan, Kansas.

The specimen reported here measured 11 cm. in total length. Other characteristics are: scolex width, 1.07 mm.; scolex length, 0.8 mm.; rostellum width, 0.31 mm.; neck width, 0.4 mm.; maximum width, 1.66 mm.; number of hooks, 40; length of large hooks, 0.14 mm.; length of small hooks, 0.09 mm.; number of suckers, 8; outside diameter of the suckers, 0.23 mm.; inside diameter of the suckers, 0.13 mm.; number of proglottids (twin segments) 103. The most posterior proglottids are in the early stages of maturity. Each segment has two genital pores, i.e., one in each "twin," which occur on opposite sides. Sections of this worm, and of the triradiate specimens, show the internal anatomy of both forms to be essentially as described by the Dobrovolnys. Each of the triradiate worms has 6 suckers and one rostellum.

In November, 1935, Mr. Leo Petri made a collection of cestodes from a dog at Garland, Nebraska, and, among others, one "unusual" specimen was found which was turned over to the writer for study. The specimen has been determined as *Mesocestoides lineatus* (Goeze). Witenberg (1934, Arch. Zool. Ital., 20: 467–508) has made a rather extended study of this genus and proposes to regard this 'large form' of *M. lineatus,* "with square or elongated mature segments as *M. lineatus* forma *lineata* (Goeze, 1782)." The worm has a total length of 51 cm., a maximum width of 2.5 mm. and proglottids to the number of 525. This find is of interest because it seems to be the first North American record of this parasite occurring as a natural infestation in the dog. Witenberg reports finding it frequently in dogs in Palestine. Schwartz (1927, Science, 66: 17–18) succeeded in producing an experimental infestation, with a Mesocestoides sp., in the dog and cat by feeding tetrathyridia larvae taken from a mongoose.

Another unpublished record of *M. lineatus* from a North American mammal has been called to my attention by Dr. H. W. Manter of the University of Nebraska, who confirms the identification by Mr. Madison Shaw of this species collected in Nebraska from a raccoon, *Procyon lotor* (Linné). This is here reported as a new host record for *M. lineatus.*—G. Robert Coatney, *State Teachers College, Peru, Nebraska.*

A NEW HOST FOR *LEUCOCHLORIDIUM DRYOBATAE*

One of my assistants, Mr. John B. Calhoun, found a trematode in the small intestine of an immature male purple finch caught at the University of Virginia

on January 30, 1936. Only one specimen was obtained from nine birds examined. Although it differs slightly in some of the dimensions, the general structure is the same as that of *Leucochloridium dryobatae* described from the hairy woodpecker, *Dryobates villosus villosus,* by McIntosh (Jour. Parasit., 19: 32–55, 1932). Mr. McIntosh has examined the specimen and concurs in this opinion. This specimen was found in the small intestine whereas McIntosh gives the cloaca as the location. In addition to a new location *L. dryobatae* is reported from a new locality and a new host, *Carpodacus purpureus purpureus.*—Bruce D. Reynolds, *University of Virginia.*

EFFECTS OF ALLANTOIN ON TRICHOMONAD FLAGELLATES IN VITRO AND IN VIVO

Recent studies reported by Robinson (Jour. Parasit., 1935, 21– 354–358) indicate that allantoin stimulates the growth of tissue. The experiments reported here were undertaken to test its effects on trichomonad flagellates. Two experiments were carried out with *Trichomonas hominis* in cultures of Ringer's solution plus 0.5 per cent Loeffler's dehydrated blood serum. In Exp. I, three sets of 10 tubes were inoculated with 10,000 *T. hominis* each; one set was kept as controls; one set was prepared with 0.5 per cent allantoin; and one set with 0.125 per cent allantoin. Two and four days later the control tubes contained large numbers of trichomonads but the allantoin tubes were negative.

In Exp. 2, three sets of 10 tubes were set up as before but a smaller amount of allantoin was used; one set was prepared with 0.05 per cent allantoin and one with 0.01 per cent allantoin. The average numbers of trichomonads per cmm. were as follows: on the second day, control 90, allantoin 0.05 per cent 12, allantoin 0.01 per cent 47; on the fourth day, control 97, allantoin 0.05 per cent 0, allantoin 0.01 per cent 95; on the sixth day, control 35, allantoin 0.05 per cent 0, allantoin 0.01 per cent 12; on the eighth day, control 5, allantoin 0.05 per cent 0, allantoin 0.01 per cent 0.5. Apparently the presence of allantoin in the quantities we used is detrimental to *Trichomonas hominis* in culture.

Three experiments were then carried out to determine whether allantoin is detrimental to trichomonads in rats when given to the host by mouth. In the first experiment 0.5 per cent allantoin in water was substituted for the drinking water of 12 rats positive for trichomonads. The rats drank the mixture as readily as they did water. After 7 days, 3 experimental rats and 3 controls were all found to be well-infected with trichomonads. Then 4 of the rats were inoculated intragastrically each with 0.5 gram of allantoin suspended in water. Four days later they were killed and found to be highly infected with trichomonads. Three of the rats were then given 3.7 grams of allantoin each in twice this weight of normal food; they were all infected with trichomonads 24 hours later. The remaining 2 rats were killed after they had been treated with drinking water containing 0.5 per cent allantoin for 30 days; they were likewise positive for trichomonads.

In the second experiment 10 trichomonad-free rats were infected with *T. hominis* intragastrically and 4 of them were then given 2 grams of allantoin in 4 grams of their regular food on 2 successive days. Two of these killed on the third day were free from trichomonads, whereas 2 controls were positive. The other 2 were killed 7 days later; one was positive and the other negative, but the 2 controls were also negative.

In the third experiment 6 rats infected with *Trichomonas parva* were used. Three of them were given 2 grams of allantoin in 4 grams of regular food for 2 successive days. At the end of 7 days one was killed; it and a control were both positive. At the end of 14 days the other 2 and 2 controls were killed; they were all positive for *T. parva.*

These data indicate that allantoin does not stimulate the growth of *T. hominis* when added to the culture medium in the quantities stated above. They also

indicate that allantoin has no apparent effect on *T. hominis* or *T. parva* in rats when given by mouth as described above.—Robert Hegner and Lydia Eskbridge, *School of Hygiene and Public Health, Johns Hopkins University.*

THE INGESTION OF THE INFLAMMATORY EXUDATE BY SWINE LUNGWORMS

The lungworms of swine (*Metastrongylus elongatus, M. salmi* and *Choerostrongylus pudendotectus*) are known to produce bronchopneumonia and bronchitis. Owing to the inflammatory processes, involving mucous degeneration and desquamation of the bronchial epithelium and a heavy leucocytic infiltration in and around the bronchi, a thick white exudate collects in the bronchial tubes. This exudate is composed, for the most part, of mucus, leucocytes, desquamated epithelial cells and varying numbers of red blood cells, the latter probably coming from the congested blood vessels in the peribronchial tissue. In areas where sexually mature worms are present, the exudate also contains large numbers of lungworm eggs. As observed in the course of post-mortem examinations, the bronchi and bronchioles are usually filled with masses of worms and the above named exudate. Hung (N. Amer. Vet., 7: 21–23, 1926) in a paper on the histopathology of *M. elongatus* infestations in swine, states that "the blood vessels between the bronchopneumonic parts are generally enlarged and contain numerous eosinophils. It is interesting that eosinophils and red blood corpuscles are frequently found in the intestines of the worms, indicating that the worms feed on blood."

The writer, however, has observed that the contents of the worms' intestine is composed of elements identical with those found in the exudate surrounding the worms. Sections of the lungs of swine naturally infested with undetermined species of lungworms and the lungs of guinea pigs experimentally infected with *M. elongatus,* were stained with Maximow's azure II-eosin and hematoxylin, following fixation in formalin. Mucus containing large numbers of eosinophilic and neutrophilic polymorphonuclear leucocytes, lymphocytes and desquamated epithelial cells was readily recognized in cross sections of the worm's intestines. (Fig. 1.) Erythrocytes were seen in the lumen of the intestine in some instances.

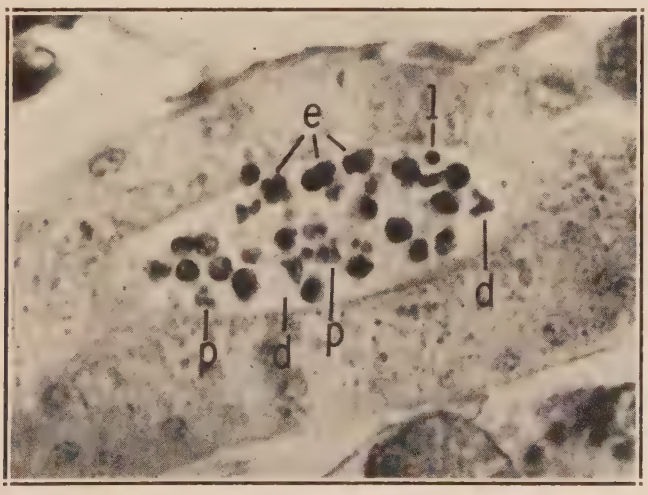


Fig. 1. Oblique cross section of intestine of *Metastrongylus elongatus* in lung of guinea pig showing intestinal contents.

e, eosinophile; p, neutrophile; l, lymphocyte; d, desquamated epithelial cell. ×600.

Some of the leucocytes and epithelial cells in the lumen of the intestine of the worms had the same appearance as those in the bronchial tissue of the host, while other cellular elements in the intestine of the worms appeared to have been digested in part. This may explain why red blood cells were seen only occasionally, since these elements are probably easily digested.

The lungworm eggs, ranging from 43 μ to 64 μ in length and from 38 μ to 45 μ in width, were never seen in the worms' intestine, probably because the size of the eggs would preclude their being swallowed. The distance between the margins of the esophageal radii where the lumen is the smallest, that is 15 μ to 20 μ posterior to the labial region, was determined from cross sections of several worms. This distance, which approximates the maximal dilation of the esophageal lumen, was found to vary from 10 μ to 18 μ. It is safe to conclude, therefore, that while the worms are able to ingest the small exudate cells they cannot ingest objects as large as their own eggs.

Since swine lungworms possess only simple lips, they are probably incapable of lacerating the tissues and obtaining blood directly. Hung's inference that the worms feed on blood is, therefore, misleading. In the light of the present writer's observations, it appears that the worms ingest the inflammatory exudate, and that the ingested red blood cells and leucocytes are merely elements of this exudate.—Dale A. Porter, *Zoological Division, Bureau of Animal Industry, U. S. Department of Agriculture.*

PLASMODIUM POLARE IN THE VERTEBRATE HOST

Plasmodium polare was first described by the author from a cliff swallow in which it was found in the summer of 1934, ("How many species of avian malaria parasites are there?" Jour. Parasit., 20: 334; Amer. Jour. Trop. Med., 15: 265–283), and has been seen since in three other birds of the same species. As far as is known at present it is restricted in nature to this species of host, for nothing like it has been reported by any other investigator, and the author has failed to find it in any other of approximately a thousand birds examined, belonging to about fifty species (and including forty-one swallows of four other species).

In the beginning it appeared to be impossible to transmit this species of malaria to the canary, although seven attempts were made and relatively large amounts of infected blood were used. After about five months of observation, however, a single parasite was found in one of these birds, and subinoculation into a clean bird resulted in a demonstrable infection two months later. Since then eight birds have been infected, and the parasite has been carried through five transfers. Inoculation was intravenous in all cases, and the inoculum was never less than 3000 cmm. of blood, with enough saline-citrate solution to prevent clotting. The incubation period has varied from about two weeks to two months.

So far all the infections have been extremely light, and it is very uncommon to find more than one or two parasites in a ten-minute examination of a single smear (using a 5× ocular and a 90×, 1.3 oil immersion objective). All the birds infected have been followed for three months or more and in no case has anything like an acute stage been observed, or a relapse in the ordinary sense of the word. The parasite-level is low to begin with and remains so, but so far no case of spontaneous recovery has been seen. The first case in which parasites were found has been under observation for six months since parasites originally appeared, and they may still be occasionally seen.

There is no evidence to indicate that this species of malaria is at all pathogenic to the canary, and it may be added that all four of the infections seen in cliff swallows were also light. The first one found was in a young bird not more than three weeks old, so that it could not have been a chronic infection, and yet parasites were relatively few in number even here. One case has been autopsied and smears made of the various organs, but no localization of parasites was found anywhere, nor were any cells other than erythrocytes infected.

Morphologically this species has been found to exhibit exactly the same characteristics in the canary as in the cliff-swallow. In the case from which it was originally described it was impossible to be absolutely sure that some of the gametocytes observed did not belong to some species of Hemoproteus, to which they often bear a striking resemblance, but inasmuch as gametocytes of the same type also occur in the infected canaries it is clear that they must have been malaria forms.

So far no evidence of periodicity in the asexual cycle has been observed, and until heavier infections are obtained such studies will be impossible. REGINALD D. MANWELL, *Dept. of Zoology, College of Liberal Arts, Syracuse University, Syracuse, N. Y.*

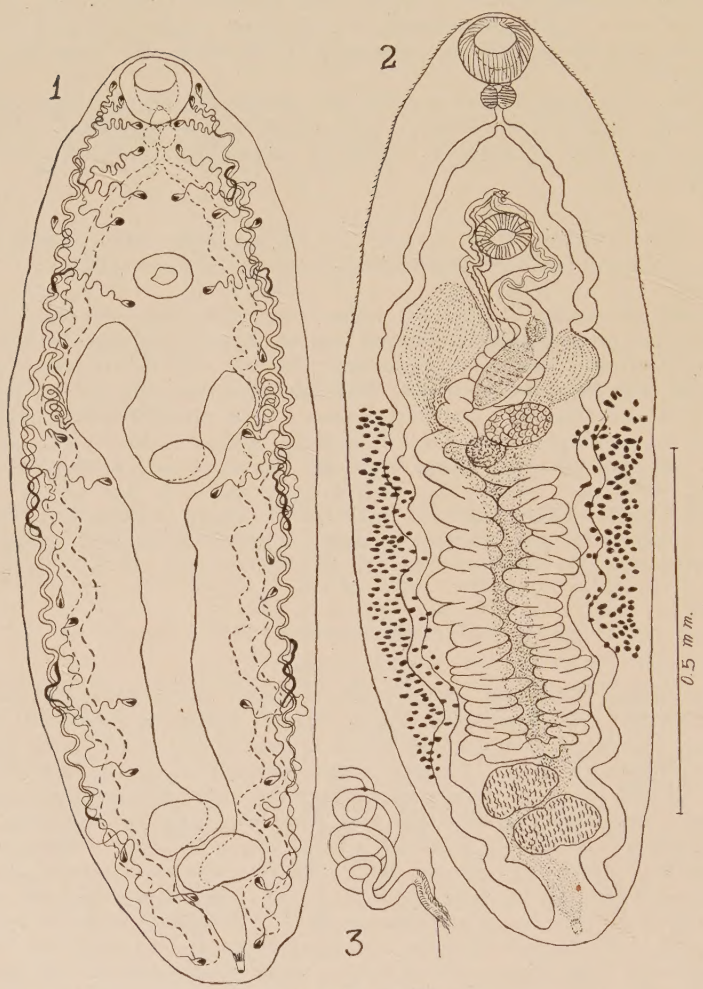
A NEW TREMATODE PARASITE FROM THE MUD-TURTLE, *KINOSTERNON SUBRUBRUM HIPPOCREPIS* (GRAY)

During the spring of 1933 the writer recovered eight specimens of what appear to be a new species of trematode from the upper small intestine of a mud-turtle, *Kinosternon subrubrum hippocrepis* (Gray), collected from the swamp just south from Raceland, Louisiana. The specimens ranged in size and development from very small and immature forms to fully matured individuals. This range in development greatly aided in working out the details of the excretory system, since the absence of eggs from the uterus in some of the individuals rendered them more suitable for making observations on this portion of the anatomy. So far as the writer is aware the following description of the excretory system is the first to appear for any member of the subfamily Telochiinae. The species is herein recorded under the proposed name of *Cercorchis kinosterni* n. sp.

Cercorchis kinosterni n. sp.

(Figs. 1, 2, and 3)

Description: Cercorchis. Body elongated oval in outline, with gently rounded extremities and slightly flattened ventral surface; measuring from 1.00 mm. to 1.78 mm. (average 1.40 mm.) long by an average width of 0.40 mm.; greatest width in region of ovary. Cuticula armed with minute spines from anterior margin to level of ovary. Oral sucker subterminal, averaging about 0.10 mm. in diameter. Acetabulum transversely oval, 0.070 mm. long by 0.095 mm. wide, lying in posterior portion of first body fourth, or 0.3 to 0.4 mm. behind anterior body margin. Prepharynx present, short. Pharynx transversely oval, 34 μ long by 43 μ wide, with few peripharyngeal gland cells. Esophagus short, 40 μ to 60 μ long. Caeca uniform, wavy, extending to level of posterior margin of posterior testis or to caudal margin of body. Ovary transversely oval, 0.07 mm. long by 0.10 mm. wide, lying slightly left of midline, close to end of cirrus sac, 0.21 mm. to 0.30 mm. behind acetabulum. Shell gland surrounding oötype. Laurer's canal and globular receptaculum seminis present. Uterus distinct, descending by compact wavy loops on left side of body to level of anterior testis, ascending by similar loops on opposite side of body to genital pore (uterus makes three or four short loops in midline in front of ovary). Metraterm well developed, about three-fifths length of cirrus sac. Ova small, 18 μ wide by 40 μ long, numerous, operculated. Vitellaria follicular, mainly lateral in position, slightly overlapping caeca, extending from level just in front of ovary to near level of anterior testis (vitelline tract more extensive on right side). Testes near posterior margin of body; anterior testis slightly displaced to right of midline, 0.07 mm. long by 0.10 mm. wide; posterior testis slightly displaced to left of midline, 0.09 mm. long by 0.12 mm. wide. Vasa efferentia uniting a short way before entering cirrus sac. Cirrus sac long, convoluted, passing either to right or left of acetabulum, extend-



EXPLANATION OF PLATE

(Figures 1 and 2 are drawn with the aid of the camera lucida.)

FIG. 1. *Cercorchis kinosterni* n. sp. Ventral view of specimen showing the details of the excretory system. Outline ×43.

FIG. 2. *Cercorchis kinosterni* n. sp. Ventral view of specimen showing the details of the internal organization. ×43.

FIG. 3. *Cercorchis kinosterni* n. sp. Diagrammatic sketch of the common collecting tubule to show the cilia in the proximal portion.

ing from genital pore to level of ovary, containing well defined vesicula seminalis, a slightly dilated pars prostatica with numerous gland cells, a long ductus, and a weakly developed cirrus. Genital pore ventral, slightly left of midline, about 45 μ in front of acetabulum, forming a very short genital sinus. Excretory system characteristic; main stem of bladder long, median, giving rise to relatively short cornua in region of ovary; cornua ending behind level of acetabulum; common collecting tubules given off from lateral margins of cornua at about midregion of their extent, containing cilia in proximal portions, giving rise to anterior and posterior main collecting tubules at about level of termination of cornua; each main collecting tubule giving rise to three accessory tubules; each accessory tubule giving rise to three capillary tubules; each capillary tubule terminating in a single flame cell: Flame cell pattern of the $2\times6\times3$ type.

Host: Kinosternon subrubrum rippocrepis (Gray).

Habitat: Upper small intestine.

Locality: North America (United States, Raceland, Louisiana).

Type specimen: Writer's private collection, Zoology Department, University of Georgia, U. S. A.

Cercorchis kinosterni perhaps is more closely related to *C. bairdi* Harwood, 1932, than to any other member of the genus. The species may be distinguished from its nearest relative by its greater width, its shorter length, the uneven distribution of the vitellaria, the more caudal position of the ovary, the extent of the cirrus sac, the arrangement of the testes, and the sizes of the various internal organs.

The writer accepts the genus *Cercorchis* Lühe (1900, Centralbl. f. Bakt. 1 Abt. Orig., 28: 555) as emended by Perkins (1928, Parasitol., 20: 236–256) and suggests that *Telorchis stenonura* Ingles (1930, Jour. Parasitol., 17: 101–103) be considered as a synonym of *Cercorchis stenonura* (Ingles, 1930). The writer also accepts the view recently advanced concerning the relationship of the Telorchiinae to the family Plagiorchiidae. Fuhrmann (1928, W. Kukenthal, Handbuch der Zoologie, Trematoda, p. 112), Perkins (1928, *Loc. cit.*), Mehra (1931, Parasitol., 23: 157–178), and McMullen (1934, Jour. Parasitol., 20: 248) have suggested that a relationship exists between these two groups of parasites; the view being supported primarily on marital characters. More recently McMullen (1935, Jour. Parasitol., 21: 217) has confirmed his views on a comparative study of the developmental stages as they are known for the two groups of worms. This view is further advanced when the details of the excretory system are taken into consideration. In this respect we might add that the excretory system in the Telorchiinae, as worked out for *Cercorchis kinosterni* n. sp., is identical to that observed for the family Plagiorchiidae with the possible exception of the presence of cilia in the proximal portions of the common collecting tubules. Whether or not this feature of the system will be found to be constant for all members of the subfamily group can only be determined after much study. The flame cell pattern of $2[(3+3+3)+(3+3+3)]$ is common to both the Telorchiinae and the Plagiorchiidae.—Elon E. Byrd, *University of Georgia.*